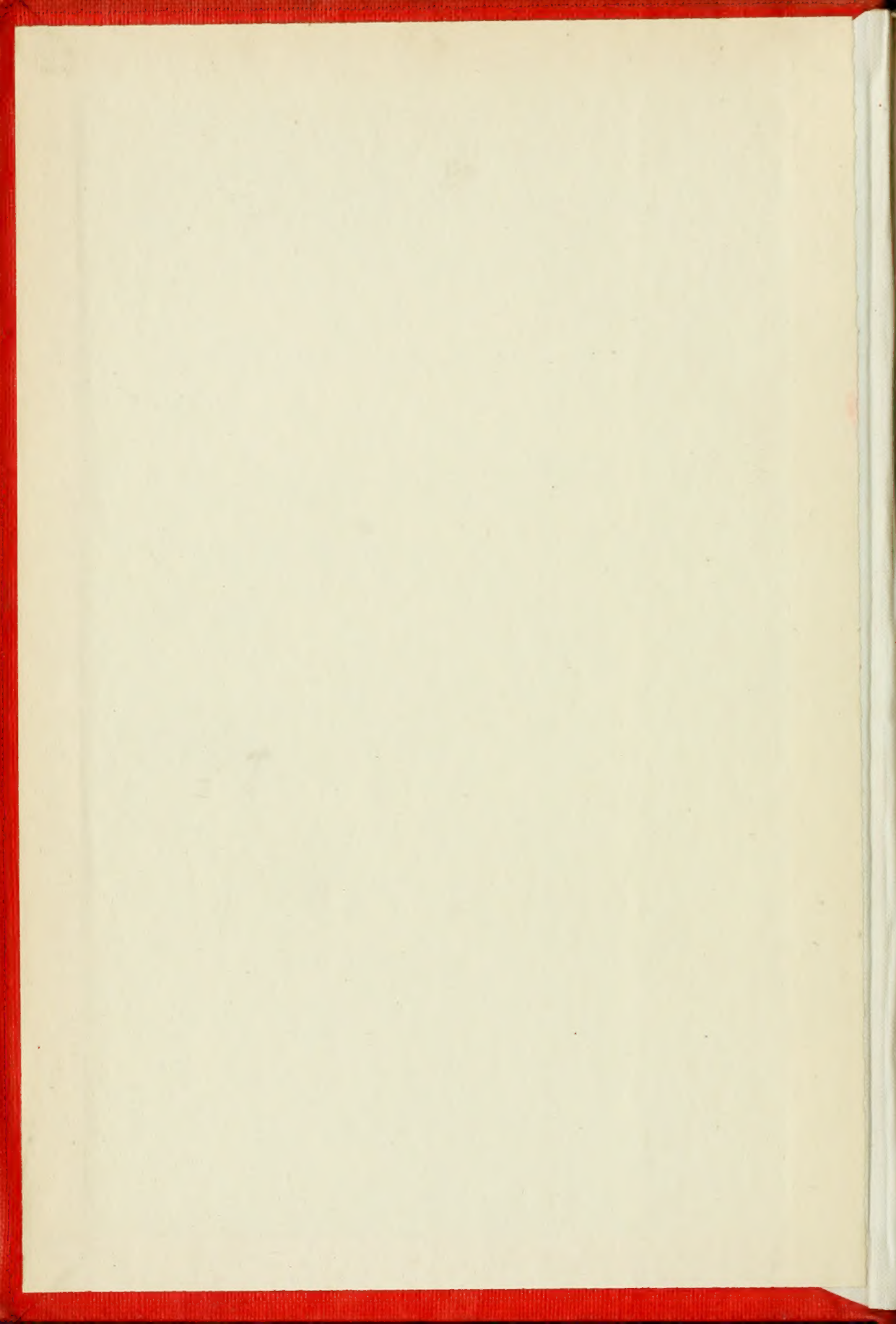


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THE MOTOR FUNCTIONS OF THE INTESTINE FROM A NEW POINT OF VIEW*

WALTER C. ALVAREZ, M.D.

SAN FRANCISCO

According to modern textbooks the small bowel is a tube whose structure and physiologic characteristics are much the same from one end to the other; and material is supposed to move aborally because of the myenteric reflex, or Bayliss and Starling's "law of the intestine." According to this law,¹ "Excitation at any point of the gut excites contraction above and inhibition below"; and it can easily be seen how this mechanism must favor the aboral progress of material through the intestine.

Cannon² has warned us, however, that this reflex and normal peristalsis are not the same, nor is it always in control of the mechanism of the bowel. A moment's thought will show that this must be true; otherwise food once introduced into the duodenum would never stop in its rush to the anus. My own graphic records of peristaltic rushes, secured with six or seven enterographs, rarely show any relaxation before the oncoming wave. On the contrary, powerful contractions appear below, which serve to stop the rush from going too far.

Unfortunately, the stimuli ordinarily used to elicit the reflex have been traumas—pinches, sudden distention by balloons, etc., and little effort has been made to see what happens above and below the normal loopful of digesting food.³ Much might be said about the

* From the George Williams Hooper Foundation for Medical Research, University of California.

* Read before the Section on Pathology and Physiology at the Sixty-Sixth Annual Session of the American Medical Association, San Francisco, June, 1915.

1. Bayliss and Starling: *Jour. Physiol.*, 1899, xxiv, 110.

2. Cannon: *Am. Jour. Physiol.*, 1912, xxx, 125.

3. "What causes the myenteric reflex to appear or not, when material is present, is as yet undetermined," Cannon: *Am. Jour. Physiol.*, 1912, xxx, 125.

difficulties met with by those who have studied this reflex; about the narrow limits (2 to 3 cm. on either side of the stimulus) within which it ordinarily is operable, but it would be beside the point. The existence of the reflex has been well established; but we do not know how far it enters into normal peristalsis, and we must admit that this explanation for the downward progress of food through the intestine is not sufficient in itself. Perhaps for this reason the "law of the intestine" has been of little help to the gastro-enterologist in the solution of his problems. "It does not govern the rhythmic contractions of the small intestine, the rhythmic peristalsis and antiperistalsis of the colon, and probably not the rhythmic waves of the stomach. . . . For these activities *the tonic contraction of the wall of the canal is all-important.*"⁴

FUNCTIONAL DIFFERENCES IN THE VARIOUS PARTS OF THE INTESTINE

A number of writers⁵ in the past have noticed differences in muscular strength and tone — differences in rhythm and irritability — in the various parts of the bowel; but they did not seem to realize the importance of their findings, and their writings certainly have had no effect on our conception of the motor functions of the intestine. My own study of these functional differences in the various parts of the intestine of animals soon suggested to me that they might play a large part in the passage of material through the tract. The contents of a muscular tube so constructed

4. Cannon: *The Mechanical Factors of Digestion*, London, 1911, p. 195.

5. For some of the literature, particularly in regard to differences in rhythm, see Alvarez: *Am. Jour. Physiol.*, 1914, xxxv, 177; *ibid.*, 1915, xxxvii, 266. Differences in muscular thickness in different parts of the small intestine have been noted by Monks: *Ann. Surg.*, 1905, xlii, 549; Bérard: *Cours de physiologie*, Paris, 1849, ii, 285; Jonnesco: Poirier et Charpy's *Traité d'anat. humaine*, Paris, 1901, iv, 284; Flint: *Textbook of Human Physiology*, New York, 1895, p. 234, and others. Differences in sensitiveness to various stimuli have been observed by Schillbach: *Arch. f. path. Anat.*, etc., Berl., 1887, cix, 281; Biedermann: *Arch. f. d. ges. Physiol.*, 1889, xlv, 372 and 379; Floel: *Ibid.*, 1885, xxxv, 161; Lüderitz: *Arch. f. path. Anat.*, etc., Berl., 1889, cxviii, 24 and 32, and others. Differences in the driving power have been shown by Hess: *Deutsch. Arch. f. klin. Med.*, 1886-7, xl, 105. A balloon in the dog's intestine was fastened to a thread which came out of a gastric fistula and ran over a pulley. At the end of the string was a little bag which could be filled with shot until its weight stopped the progress of the balloon. Eighteen cm. from the pylorus 228.5 gm. were needed; 38 cm. from the pylorus 90.4 gm. were needed; 50 cm. from the pylorus 75.5 gm. were needed.

would probably move from the more active, irritable and powerful regions above to quieter, less sensitive segments below, where the muscular coats are thinner and less tonically contracted. Reverse peristalsis would hardly occur unless the lower parts of the bowel could be stimulated to an activity greater than that ordinarily found in the upper stretches.

We think of the heart today, not as pump with purely mechanical valves, but as an elaboration of the primitive tube in which the rhythmic power of each segment varies inversely as does the distance from the sinus.⁶ So important is this difference that the current is reversed in the simpler types of heart if the aortic end be made to beat faster.⁷

Similar experiments have been performed with segments of the digestive tract. The direction of peristalsis in the crop of *aplysia*, a large marine snail, can be changed at will by increasing the tension at one end or the other.⁸ Cannon⁹ has shown that waves will course over the excised stomach of a frog or cat in either direction, depending on the location of a pulsating tonus ring. Still more pertinent is the experiment in which he dipped the aboral end of a short loop of small intestine into a weak solution of barium chlorid, and by thus raising its tone, reversed the direction of peristalsis.¹⁰ Stimuli which raised the tone in the middle of such a loop sent off waves in both directions.¹¹

The few references I have been able to find to the motor functions of the digestive tract in the lower forms of life show how much the progress of food through their simple tubes depends on local differences in tone and rhythmicity.¹² It is harder to recog-

6. Erlanger: Arch. Int. Med., 1913, xi, 334.

7. Gaskell: Schafer's Textbook of Physiol., London, 1900, ii, 184.

8. Von Brücke: Arch. f. d. ges. Physiol., 1905, cviii, 209.

9. Cannon: Am. Jour. Physiol., 1909, xxiii, xxvii.

10. Cannon: Am. Jour. Physiol., 1912, xxx, 119.

11. Cannon: Am. Jour. Physiol., 1909, xxiii, xxvii. "A ring at the cecum repeatedly sends off downward running waves; a new ring made now near the terminus of these waves starts reversed waves; and a tonic ring made midway in the proximal colon not infrequently will originate waves which pass away in both directions." Cannon: Arch. Int. Med., 1911, viii, 419.

12. See papers by Bottazzi on the *Aplysia*, a large marine slug: Jour. Physiol., 1897-98, xxii, 481, and v. Brücke: Arch. f. d. ges. Physiol., 1905, cviii, 202; Bottazzi on some fishes, Ztschr. f. Biol., 1902, xliii, 364, and Hardy and McDougall on lower crustaceans, Proc. Cambridge Philosoph. Soc., 1893, viii, 41. A valuable point brought out in these studies and, I believe, largely applicable to man, is that material does not press up to and pack against the sphincters. The real barrier is

nize this simple mechanism in the much elongated and more specialized gut of a mammal, but there is considerable evidence that short nerve paths through Auerbach's plexus, and longer paths through the mesentery and ganglia keep the stomach and cecum as close together, functionally, as they are in a snail or a frog.¹³

CLOSE CONNECTION OF VARIOUS PARTS OF TRACT

The use of a number of enterographs at one time, particularly in the cat, has shown me how immediate is the response of one part of the tract to changes in tone and activity in another. In addition, what might be called ripples, invisible to any one watching the bowel, can plainly be seen on the records, running in a few seconds from the pylorus to the colon. Now and then these ripples show themselves as short, diastaltic rushes, much perhaps as a swell from the ocean striking a shallow shelf suddenly becomes a comber. Without the graphic record no one would think of associating a short rush 200 cm. from the pylorus with a particular gastric contraction or with a sudden cessation of colonic activity. Bayliss and Starling have well said that, "every point of the intestine is in a state of activity, which can be played upon and modified by impulses arriving at it from all portions of the gut above and below."¹⁴ The promptness with which a mouthful of food introduced in the stomach causes material to pass through the ileocecal valve¹⁵ or to

not the sphincter, but a ring of muscle with high tone and inherent rhythmicity, placed around or just above the valve. This not only blocks impulses coming down, but starts up reverse waves that protect the sphincter from being put under pressure. I have shown how the sacculus rotundus may protect the ileocecal sphincter of the rabbit, Alvarez: *Am. Jour. Physiol.*, 1914, xxxv, 184. A similar thickening has been described in man by Keith: *Jour. Anat. and Physiol.*, 1903, xxxviii, 7, and Elliott: *Jour. Physiol.*, 1904, xxxi, 158. The greater tone of the lower end of the colon in man serves the same purpose, probably, as do the pulsating tonus rings that guard the anus in the above-mentioned fishes and crustaceans.

13. Roith describes two cases in which rectal injections brought about movements in the upper colon although the bowel in between was completely severed. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv, 208. Marbaix produced inhibition of gastric emptying by filling the jejunum, even after complete section of the duodenum, *La Cellule*, 1898, xiv, 283. Surmont and Dubus obtained immediate responses from the colons of dogs and cats by stimulating the duodenum, even after removing sections of ileum, *Arch. d. Mal. l'App. dig.*, 1912, vi, 181.

14. Bayliss and Starling: *Jour. Physiol.*, 1899, xxiv, 116.

15. Hertz: *Jour. Physiol.*, 1913, xlvii, 55. I have observed the promptness of this reflex many times. Cole's observation—see note 66—is also very striking.

rush through the colon,¹⁶ shows that the human intestine also acts very much as a short unit.

THE THEORY

Returning, then, to the idea of a tube with greater tone and rhythmicity at the oral end, it is apparent that a rise of tone at the upper end, due to the introduction of food or to irritating lesions, would hurry the aboral progress of food; while a similar rise at the lower end should slow the current or even reverse it. A rise anywhere in the middle of the tube should cause material to flow both ways from that point.

A more rapid current might be brought about also by lowering the tone of the lower end.¹⁷ If the gradient of forces should become a horizontal line, no progress could be made, no matter how great the activity throughout the tube.¹⁸

While much work yet remains to be done on animals before such a theory can be firmly established, it seems worth while, at this time, to go over the literature of the laboratory worker, the clinician, the surgeon, and particularly of the radiologist, to see how well this hypothesis accords with the facts already at hand. Physiologic methods (Roentgen ray, gastric analysis, etc.) in the hands of the clinician have been yielding results that are not to be despised even if Dame Pathology did arrange the experiment; and an ill-assorted collection of observations awaits only the systematizing touch of a basic theory.

That the rhythmic segmentation of food anywhere in the tract is associated with a rise of tone has been

16. Meyer-Betz has seen the colon empty into the ampulla almost immediately after the patient took some food. Immediate postprandial desire for stool, or even diarrhea in children and some adults, is well known. *München. med. Wochenschr.*, 1911, lxx, 1717.

17. Material seems to go through the small intestine faster than usual when there is a large fistula in the terminal ileum or cecum. There seems ordinarily to be a retarding influence from the highly tonic colon. See Demarquay: *L'Union med.*, 1874, xviii, 906; and Macewell: *Lancet*, London, 1904, ii, 1298.

18. Katsch concludes that there is no proportion between the intensity of peristalsis and the progress of material through the tract. At times the food will move with ease through long stretches of an almost passive bowel, and yet the violent contractions produced by drugs like pilocarpin may have little forwarding effect. *Ztschr. f. exper. Path. u. Therap.*, 1913, xii, 287 and 233. Bayliss and Starling, *Jour. Physiol.*, 1900, xxvii, 126, and Kelling, *Ztschr. f. Biol.*, 1903, xlv, 240, have shown that the waves frequently put no pressure on the contents of the gut.

well proved.¹⁹ That irritating lesions also raise the local tone is the daily experience of the radiologist. The spastic pylorus or hour-glass stomach with adjacent ulcer, and the hypertonic stomach and duodenum in duodenal lesions bear witness to this point.²⁰

The word tone may have to be used rather loosely in this paper for the sake of brevity. We cannot tell yet what part other factors — such as differences in rhythm and irritability — play in altering the gradient of forces through the tract.²¹ The words higher and lower, which will appear frequently in the following pages, refer to differences in tone and muscular force and have absolutely no relation to position in the abdomen, or to gravity. I trust no one will misunderstand or forget this point. There are enough surgeons already who tinker at the bowel as if it were a coil of rigid tubing always held in one position. The intestine is a muscular organ and "material does not move along the canal unless the pressure is greater on one side of it than on the other."²²

THE EFFECTS OF FOOD INTRODUCED AT THE UPPER END OF THE TRACT

It may sound like a Hibernianism, but food does go down the tract more easily because it is put in at the upper end. The rise of tone there probably makes the gradient of forces steeper down the intestine.²³ A contrast meal will go down the bowel faster if followed by more food a few hours later.²⁴ This second meal

19. Cannon: *Arch. Int. Med.*, 1911, viii, 421. Mall: *Johns Hopkins Hosp. Rep.*, 1896, i, 45. "If the intestine is at rest it is anemic, small and long; when digesting it is hyperemic, large and short." I have placed two markers 3.5 cm. apart on an active and apparently stretched piece of bowel and have found them 8 cm. apart later when this region had emptied and quieted down.

20. "I have always believed that the severity of the spasm resulting from an ulcer was dependent on its irritability and not on its size." Barclay: *Arch. Roentg. Ray*, 1913, xviii, 235. Schmieden gives it as a rule that, in ulcer, the healthy parts of the stomach contract; in carcinoma, the diseased parts. *Arch. f. klin. Chir.*, 1911, xcvi, 265.

21. "The tonic state is fundamental. I have repeatedly attempted to call forth rhythmic contractions, both in the atonic colon and in the atonic stomach, by distention, but without success. The tonic state is therefore quite as important as the internal pressure; indeed, it is the condition for the existence of that pressure." Cannon: *Am. Jour. Physiol.*, 1911, xxix, 242. See also *Arch. Int. Med.*, 1911, viii, 417.

22. Cannon: *Mechanical Factors of Digestion*, London, 1912, p. 76. According to Keith, there is enough muscular tissue in the colonic wall of man to form a mass as large as the biceps of a blacksmith's arm. *Brit. Jour. Surg.*, 1915, ii, 584.

23. During active digestion, the gradient of rhythm is made steeper by a rise in the duodenal region and a fall in the ileum. Alvarez: *Am. Jour. Physiol.*, 1915, xxxvii, 277.

24. Hertz: *Arch. Roentg. Ray*, 1912, xvii, 216.

probably keeps the gradient steeper than usual by maintaining the high level of the gastric tone.

There is some evidence that pleasurable psychic stimuli still further raise this tone. Several observers have noticed that contrast meals leave the stomach more rapidly if they are made palatable.²⁵ "Sham drinking," particularly in thirsty animals, will cause water to leave the stomach of a dog much faster than it otherwise would.²⁶ It will also prevent the back-flow into the stomach which occurs when fat is put into the duodenum through a fistula.²⁷ The latter observation is explained if the pleasure of drinking has raised the tone of the stomach above that of the duodenum.

It is a common observation that patients who have vomited albumin water and other bland liquids for days may cease when given solid food. Good nurses are well acquainted with this trick and often stop post-anesthetic vomiting with a little dry toast. Attacks of "cyclic" vomiting in children may also be stopped in this way.²⁸ I hope later to present evidence that vomiting is often a manifestation of reversed currents due to the presence of an abnormally high tone in the jejunum.²⁹ Water would probably not alter these conditions enough, as it passes rapidly into the intestine

25. Hertz: Arch. Roentg. Ray, 1912, xvii, 219. See also Haudek and Stigler, who speak of slow gastric emptying when the food is taken without appetite, Arch. f. d. ges. Physiol., 1910, cxxxiii, 159. Also Gilmer: Centralbl. f. Roentgenstr., etc., 1911, ii, 235. Takahashi showed that cats' stomachs emptied half as fast when the animals were fed with a spoon as when they ate by themselves. Arch. f. d. ges. Physiol., 1914, clix, 389. Sailer believes that the bismuth meal leaves the stomach quicker if the patient drinks it than if it is put in by a tube, Univ. Penn. Med. Bull., 1906, xix, 136.

26. Best and Cohnheim: Ztschr. f. physiol. Chem., 1910, lxi, 116. The dog has an esophageal fistula in the neck so that water or food swallowed does not reach the stomach, hence the term "sham-feeding."

27. Best and Cohnheim: Ztschr. f. physiol. Chem., 1910, lxi, 125.

28. McClure: Am. Jour. Dis. Child., 1914, vii, 48. Hahn: Med. Klin., 1911, vii, 1452. Tobler: Deutsch. med. Wchnschr., 1914, xl, 1042. Bartlett: Am. Jour. Dis. Child., 1914, vii, 310.

29. I have graphic records from cats, and from a man with a stomach, showing that a marked rise in the tone of the jejunum preceded spontaneous vomiting. Strange to say, almost all writers on vomiting have apparently forgotten that there is such a thing as an intestine. Physicians think they can stop vomiting if only they can keep food out of the stomach. Cohnheim and Dreyfus produced nausea and vomiting in dogs repeatedly by distending the small intestine with a balloon. Ztschr. f. physiol. Chem., 1908, lviii, 56. Hirsch produced vomiting in dogs by giving solutions of organic acids. Acetic acid was most active in this regard, apparently because it had the least effect on the stomach and the most marked stimulating effect on the intestine. This produced back currents as shown by the flow of bile into the stomach. A weaker impulse, as from lactic acid, caused only a delay in gastric emptying. Centralbl. f. klin. Med., 1893, xiv, 380. That nausea is associated with a drop in gastric tone has been observed by Barclay: Brit. Med. Jour., 1910, ii, 539.

without having much effect on gastric tone. Solid food, however, can raise the tone of the stomach sufficiently to restore the normal gradient and to send the currents downward again.³⁰

Cannon stated clearly in 1906 that "in whatever manner the pylorus may act under normal conditions, that action certainly can be overcome in abnormal states."³¹ The other factors may simply modify the chemical control. For instance, increased pressure from above, brought about by a pleasant psychic influence,³² or by distending the stomach,³³ will cause the pylorus to open before the chemical conditions are right. Cohnheim and Dreyfus³⁴ found, in a dog, that when little food was present, 10 c.c. of stomach contents injected into the duodenum closed the pylorus for from ten to fifteen minutes; but when the stomach was full of fluid, the same stimulus stopped the flow for from only one to one and one-half minutes. The distention of the duodenum by a balloon,³⁵ the irritation of the bowel by strong saline solutions,³⁶ or the simple presence of neutral food in the upper half of the small intestine³⁷ will retard by hours the emptying of the stomach.

As Cannon reminds us, many of these observations can have little to do with physiology: food ordinarily shoots through the duodenum without distending or irritating it very much, but I believe they have much

30. After the stomach has been quiet for some time with bismuth water, I have often seen it contract and start up active peristalsis immediately after the patient had eaten a small cracker.

31. Cannon: *Am. Jour. Med. Sc.*, 1906, cxxxi, 569.

32. Cohnheim and Dreyfus: *Ztschr. f. physiol. Chem.*, 1908, lviii, 57.

33. Marbaix found that 250 c.c. of water left his stomach twice as fast if he put in 250 c.c. of air on top of it. *La Cellule*, 1898, xiv, 280. Rieder comments on the more rapid emptying of the full stomach. *München. med. Wchnschr.*, 1904, li, 1550. Turck marvels that a small test-breakfast will remain in an atonic stomach longer than will a large Leube meal, *THE JOURNAL A. M. A.*, 1904, xlii, 815. Lüdin found that food taken after a contrast meal may hasten gastric emptying. *Deutsch. med. Wchnschr.*, 1913, xxxix, 1239. See also Moritz: *Ztschr. f. Biol.*, 1901, xlii, 585.

34. Cohnheim and Dreyfus: *Ztschr. f. physiol. Chem.*, 1908, lviii, 58.

35. Tobler: *Ztschr. f. physiol. Chem.*, 1905, xiv, 185.

36. Cohnheim and Dreyfus: *Ztschr. f. physiol. Chem.*, 1908, lviii, 58. Four per cent. solutions of sodium chlorid and magnesium sulphate put into the upper bowel through a fistula caused diarrhea with marked slowing of gastric emptying, nausea and even vomiting.

37. An introduction to the literature on this subject may be had through the articles of Best and Cohnheim: *München. med. Wchnschr.*, 1911, lviii, 2732. Marbaix: *La Cellule*, 1898, xiv, 268. V. Mering: *Verhandl. d. Congr. f. inn. Med.*, 1893, p. 476. Hirsch: *Centralbl. f. Klin. Med.*, 1893, xiv, 377. Baumstark and Cohnheim: *Ztschr. f. physiol. Chem.*, 1910, lxv, 484. Baumstark: *Ztschr. f. physiol. Chem.*, 1913, lxxxiv, 437. Thomsen: *Ibid.*, p. 425.

to do with pathology; and are of particular significance to those of us who, as clinicians, are dealing with abnormal states. It is unfortunate that all the efforts to explain the peculiarities of gastric emptying with gastric and duodenal ulcer and with carcinoma have been based on the chemical reflex alone. Von Bergmann and others have shown how impossible it is to trace any connection between the varying rates of emptying and the degrees of acidity in the individual cases.³⁸ We know also that the stomach gets along very well with complete achylia; and, although the emptying is a little more rapid, the intermittent spurts are very similar to those seen under normal conditions.

The slowing of gastric emptying by distending, irritating, or putting food into the bowel is explainable if we think of the intestinal tone as having been raised above that of the stomach. When the bowel empties and its tone falls, the stomach can empty again. The physiologic studies have not left it entirely clear as to whether the delay in gastric emptying is due to an inhibition of peristalsis or to pyloric spasm. A similar confusion is found in the clinical literature on six-hour stasis, where the terms pylorospasm, pyloric stenosis and gastric atony have been used almost interchangeably. Only a few men have observed closely enough to note that the stomach often fails to empty in the presence of active and powerful peristalsis, even when the pylorus is easily patent, as shown by palpation under the screen.³⁹ Such a condition is explainable if the tone of the intestine is equal to that of the stomach. Material may then churn back and forth; the pylorus may be open, and yet no progress can take place.⁴⁰

38. Von Bergmann, G.: *Centralbl. f. Roentgenstr.*, etc., 1913, iv, 3. See also Faulhaber and v. Rehwitz: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1914, xxviii, 192.

39. Holzknecht and Haudek: *Fortschr. a. d. Geb. d. Roentgenstr.*, 1914, xxi, 634.

40. Cannon and Blake state clearly that after pyloroplasty the function of the pylorus is taken over by the active contractions of the duodenum, *Ann. Surg.*, 1905, xli, 708. Dagaew removed the powerful pyloric end of the stomach, expecting that this would allow the food to run promptly into the bowel. Just the reverse happened; there was marked delay which seemed to be due to the lack of pressure from above to overcome the resistance of the active intestine. As we should expect, the usual regurgitation into the almost empty stomach was much exaggerated. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxvi, 179. Marbaix speaks of the contest between the stomach and intestine, *La Cellule*, 1898, xiv, 283. The larva of *Ptychoptera contaminata* has no pyloric valve, so van Gehuchten says the small intestine, in all its length, takes up the rôle of sphincter. *La Cellule*, 1890, vi, 277.

This balancing of forces between the stomach and intestine is probably at the bottom of many well-known phenomena. The ill effects on some people of eating between meals may be due to the slowing of the gastric emptying caused by the presence of the previous meal in the small intestine. In order to show duodenal stasis, Jordan⁴¹ gives a bismuth meal an hour or so after the patient has eaten his dinner. By that time the tone of the intestine has been raised by the presence of food and the contrast meal is held back when it reaches the duodenum.

It has been observed repeatedly that, at the close of gastric digestion, when the tone of the intestine is still high and that of the stomach has probably fallen, duodenal contents flow back into the stomach. This is particularly the case when much fat has been eaten.⁴² The explanation for this lies probably in the fact that while the tone and motility of the stomach are markedly impaired in the presence of oils,⁴³ the intestine may, at the same time, be even stimulated by the fatty acids split off in pancreatic digestion.⁴⁴

It is a common observation that a stomach will empty quite rapidly to a certain point, after which there is little change for hours. At first, through its greater tone or better reaction to distention, the stomach is probably stronger than the intestine. Later, the actively digesting bowel becomes too strong for the half-empty stomach, and there is little change until the intestine empties, or more food is put into the stomach.

41. Jordan: Arch. Roentg. Ray, 1913, xviii, 231. He has seen strong contractions forcing the contents back into the duodenum. He told Barclay that, under ordinary conditions, he does not see such stasis. "The Stomach and Esophagus," London, 1913, p. 57. Hess found, in one experiment, that a balloon put through a fistula into the duodenum of a dog did not go forward as usual. This apparently was accounted for by the presence of food in the ileum at the time. Deutsch. Arch. f. klin. Med., 1866-7, xl, 105.

42. Boldireff states that in animals that have been fasting for some time, the intestinal juice will run out of a gastric fistula continuously. This back-flow can be accentuated by giving fatty acids and fats or by injecting some 0.1 per cent. HCl into an intestinal fistula. Centralbl. f. Physiol., 1904, xviii, 457. Rehfuss, Bergheim and Hawk found bile present in the fasting stomachs of most of the normal students studied. THE JOURNAL A. M. A., July 4, 1914, p. 11. Years ago Kussmaul said "Offenbar gelangt die Galle viel leichter in den leer als in den vollen Magen." Samml. klin. Vortr., Inn. Med., 1880, No. 62, p. 1651.

43. V. Tabora says the stomach may be quiet for hours when full of fat. Centralbl. f. Roentgenstr., 1911, ii, 247. Cannon says the waves were a little slower and shallower. Am. Jour. Physiol., 1907, xx, 315.

44. Bokai: Arch. f. exper. Path. u. Pharmakol., 1887, xxiv, 158. For further discussion of this subject and references to the literature see Cannon: Am. Jour. Physiol., 1907, xx, 315.

Anything that tends to keep up the tone of the intestine will exaggerate this type of gastric emptying. I have seen it frequently in those cases, particularly of appendicitis, in which the ileum empties very slowly. This so-called "late pylorospasm" is quite characteristic also of duodenal ulcer.⁴⁵ There, I believe, the increased tone of the stomach, plus the stimulus of food, overcomes the hypertonic duodenum and brings about rapid emptying at the beginning. After this, a small residue remains in the stomach for hours, or until the next meal, presumably because the ulcer keeps the duodenum from relaxing to its proper tone level, below that of the stomach.⁴⁶

INTRODUCTION OF FOOD AT THE LOWER END OF THE TRACT

According to the theory, food introduced at the lower end of the tract should retard the progress of material coming down from above and might even reverse the current. There is ample proof in the literature that both these things happen. Clinically, it is well known that an enema given shortly after a meal is likely to cause colic, nausea, and, in sensitive people, even vomiting.⁴⁷ Radiologists know that enemas must not be given after a bismuth meal if they are to tell anything about the emptying time of the stomach or the rate of passage through the small intestine.

The few times I have ordered rectal feeding I have had to give it up on account of nausea and vomiting, which ceased only when the enemas were stopped. To illustrate: A woman with a blood pressure of 220 mm. of mercury and no clinical or radiologic signs of ulcer, before or since, had been passing tarry stools for several days. Rectal feeding and a Murphy drip were started, and only a little water was given by mouth. Fearful nausea began, and soon she was vomiting large amounts of blood-stained fluid. No blood reached the

45. CATERALL: *THE JOURNAL A. M. A.*, March 28, 1914, p. 980.

46. This may be the explanation for Cannon and Murphy's observation that food did not begin to leave the stomach for five or six hours after section and suture of the duodenum, some 10 inches below the pylorus. *Am. Jour. Med. Sc.*, 1906, cxxxi, 569.

47. Trousseau and Sydenham have discussed this point. The latter warns us that clysters become emetics in cases of ileus and typhoid where there is already a tendency to a reversal of intestinal action. *Clin. méd.*, Paris, 1865, iii, 28; Sydenham Soc. Edit., London, 1848, i, 67, 68 and 194.

anus while she was being fed per rectum. The vomiting became so serious that we then left the rectum entirely alone and gave her solid food. Nausea and vomiting stopped with the first mouthful and did not return. Tarry stools appeared again. Apparently the blood, oozing somewhere in the middle of the digestive tract, could be sent in either direction by raising the tone of either end.

Rolleston and Jex-Blake⁴⁸ collected ninety-six cases of gastric ulcer fed per rectum alone and found that twenty-six patients vomited on one or more occasions. They noticed that the giving of cleansing enemas to these rectally fed patients frequently produced nausea and retching, and, in a few cases, it brought on vomiting. There are enough cases in the literature in which the enemas were actually vomited, or in which the patient objected to the constant taste of the peptonized foods.⁴⁹

Several observers have noticed an increased tendency to colonic anastalsis after the giving of enemas.⁵⁰ The Roentgen ray has shown how frequently enemas will pass through the ileocecal valve and run up the small intestine even to the stomach. As would be expected from the theory, this is more likely to occur when some lesion in the colon has already raised the tone of the lower end of the tract.⁵¹

FOOD PUT INTO THE MIDDLE OF THE BOWEL TENDS TO MOVE BOTH WAYS

My experiences with jejunal feeding have not been entirely satisfactory on account of the nausea and

48. Rolleston and Jex-Blake: *Brit. Med. Jour.*, 1903, ii, 68. Bine and Schmoll have given up rectal feeding on account of such experiences, *California State Jour. Med.*, 1914, xii, 364.

49: Parkes-Weber: *Brain*, 1904, xxvii, 170, and Langmann: *Jacobi Festschr.*, New York, 1900, p. 375, give a good introduction to the literature on this subject.

50. Cannon: *Mechanical Factors of Digestion*, p. 150. In one cat an enema of warm water caused continuous anastalsis in the colon for one hour and twenty minutes. Ordinarily such waves appear from time to time only. See also Nothnagel: *Beitr. z. physiol. u. path. d. Darmes*, Berl., 1884, p. 20.

51. Case finds incompetency of the ileocecal valve associated most frequently with the increased antiperistalsis of hypertonic constipation, and with obstruction and disease of the colon. The incompetence of the valve is not a disease in itself; it is secondary, and due to a loss of tone in the muscle making up the valve. He quotes a number of authors who agree with him. *Arch. Roentg. Ray*, 1915, xix, 380 and 383.

vomiting which occurred from time to time.⁵² Food put in at the fistula has even been returned by mouth; and in most of the cases the discomfort could be relieved only by simultaneous mouth feeding. Here again vomiting was stopped probably by raising the tone of the stomach to its normal position above that of the jejunum. Nausea, vomiting and pain were present in another case until a simultaneous Murphy drip of glucose and salt solution was stopped, and some food given by mouth. The patient had no further trouble until one day when he had a cleansing enema.

It is common knowledge that vomiting is often associated with diarrhea. A small boy who has eaten all the blackberries he can pick is likely to vomit three quarters of the amount some hours later; and shortly after, the remainder is voided in loose movements. Presumably, enough of the irritant mess gets into the jejunum at the start to prevent the stomach from emptying.⁵³ Finally the tone becomes high enough in the upper bowel so that it can clear itself both ways. Strong psychic stimuli may bring about diarrhea and vomiting in the same way, if they raise the tone of the upper jejunum at the point where it is so richly supplied with vagus branches.⁵⁴

IRRITANT LESIONS

The motility of the tract is affected by irritating lesions, much as the theoretic considerations would lead us to expect. One of the best roentgenologic signs of duodenal ulcer is the presence of the head of the bismuth column in the descending colon after six hours. This is not pathognomonic of ulcer, but points to some irritation of the duodenum or the closely related gall-bladder and pancreas.

It appears also with hypertonic stomachs, due in some cases apparently to vagus disturbances. Another factor in the causation of this hypermotility is the rapid

52. Kelling speaks of the stagnation of food in the stomach, feeling of fullness and tendency to vomiting in jejunal feeding. *Ztschr. f. Biol.*, 1903, xliv, 248. "In the majority of cases, direct nutrition through the duodenum is a delusion. The chyme, or the greater part of it, is regurgitated into the stomach, as can be demonstrated fluoroscopically"; *Therapeutics of the Gastro-Intestinal Tract*, Wegele, Gross and Held, New York, 1913, p. 272.

53. Baumstark has shown that fermented food put through fistulas into various parts of the intestine will greatly slow the emptying of the stomach: *Ztschr. f. physiol. Chem.*, 1913, xxxiv, 437.

54. Alvarez: *Am. Jour. Physiol.*, 1915, xxxvii, 276.

outpouring of food from the stomach in duodenal ulcer, achylia gastrica and some cases of gastro-enterostomy.⁵⁵ The greater distention alone may stimulate the upper bowel to greater activity, much as a large enema has more effect than a small one.

The problems of motility with lesions at the upper end of the tract are complicated by the fact that the whole region from cardia to jejunum seems to have its tone raised by a lesion in the duodenum, or in the embryologically related bile passages, liver and pancreas. This is shown by the hypertonic stomachs and the hour-glass contractures so frequently seen in these cases.⁵⁶

The reaction of the stomach to ulcer is also complicated by so many factors that I will not try to discuss the subject at this time. The stomach is often atonic and quiet with pyloric ulcer;⁵⁷ possibly the waves are blocked by the tonus ring caused by the lesion. Actual reverse waves are often seen, particularly when the bismuth is taken at a time when a previous meal is in the intestine.⁵⁸ Under these conditions, as we have seen, there is a considerable tendency to back-flow at the upper end of the tract.

That gastric anastalsis is seldom seen with duodenal ulcer⁵⁹ is due probably to the almost complete break in continuity of the muscular coats at the pylorus.⁶⁰ This barrier deserves more study, as it may be of immense importance to us in blocking undesirable waves coming up from the intestine, and in protecting the bowel from undue influence from the powerful pyloric portion of the stomach.

55. Hertz: *Ann. Surg.*, 1913, lviii, 466, and Moynihan: *Abdominal Operations*, Philadelphia, 1906, p. 221, have seen even fatal diarrhea after gastro-enterostomy when the stomach emptied too rapidly.

56. Hertz: *Guy's Hosp. Rep.*, 1907, lxi, 403. Case: *Jour. Michigan State Med. Soc.*, 1913, xii, 577. Carman: *THE JOURNAL A. M. A.*, March 28, 1914, p. 980. Eisler: *Centralbl. f. Roentgenstr.*, etc., 1912, iii, 320. Schlesinger: *Berl. klin. Wchnschr.*, 1912, xlix, 1223. Holzknecht and Luger: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxvi, 669.

57. Barclay says when he cannot provoke any gastric peristalsis he always suspects a lesion at the pylorus, *Brit. Med. Jour.*, 1910, ii, 539. Carman: *THE JOURNAL A. M. A.*, March 28, 1914, p. 980. Kreuzfuchs believes that hypertonus of the pyloric end causes hypotonus of the body of the stomach, *Wien. med. Wchnschr.*, 1912, lxii, 1073. The blocking of waves by a tonus ring is described by Cannon: *Mechanical Factors in Digestion*, p. 188.

58. Haudek: *Wien. med. Wchnschr.*, 1912, lxii, 1061.

59. Once in ninety cases, Haudek: *Wien. med. Wchnschr.*, 1912, lxii, 1061. Carman has never seen it. *THE JOURNAL A. M. A.*, March 28, 1914, p. 980.

60. Cunningham: *Tr. Roy. Soc. Edinburgh*, 1905-7, xlv, 9. Brinton: *Diseases of the Stomach*, London, 1859, p. 268.

Jonas has shown that just as intestinal hypermotility and even diarrhea may appear when the stomach empties too rapidly, hypomotility of the bowel and constipation are seen with lesions interfering with the opening of the pylorus. He speaks of the stomach as the "*Haupt-motor*" for the intestine.⁶¹

Lesions in the course of the bowel, such as tuberculous ulceration, render the affected area so irritable that the bismuth meal goes through very rapidly, and this region appears to be empty.⁶² The bowel is often empty for some distance above an obstruction, showing that there is a powerful repelling effect.⁶³ For the same reason, in appendicitis, the last few coils of ileum may be empty; or the lower limit of the bismuth, instead of showing a bulging shoulder, tapers to a point. The obstruction is not mechanical, it is dynamic. It is becoming generally recognized that a kink in the bowel has little effect unless it becomes irritated in some way. The marked differences in severity, amount of vomiting, etc., between acute ileus and that arising slowly may depend partly on the degree to which the cause of obstruction irritates the bowel, raises the local tone and reverses the current.⁶⁴

61. Jonas: Arch. f. Verdauungskr., 1912, xviii, 769. Agéron holds similar views, *ibid.*, 1911, xvii, 584. Dagaew found that food reached the lower ileum in thirteen hours instead of six after removal of the strong pyloric muscle. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1913, xxvi, 179. Cohn found in a case of complete removal of the stomach and vagus endings that food went very slowly through the bowel, reaching the cecum only after twenty-four hours. Berl. klin. Wchnschr., 1913, l, 1393. Cole has noticed that the cecum fails to empty for a long time if the patient does not eat after the contrast meal, Am. Jour. Med. Sc., 1914, cxlviii, 110.

62. Stierlin: Centralbl. f. Roentgenstr., 1911, ii, 254. Case describes the emptiness of the cecum in cecal tuberculosis. Arch. Roentg. Ray, 1915, xix, 381. Kienbock discusses the subject at more length in Fortschr. a. d. Geb. d. Roentgenstr., 1913, xx, 231. Nothnagel says food is rushed through sections of bowel that have been inflamed and made irritable by the injection of concentrated salt solutions. Beitr. f. Physiol. u. Path. d. Darnes, 1884, p. 39.

63. Kirstein: Deutsch. med. Wchnschr., 1889, xv, 1000. Kelling: Ztschr. f. Biol., 1903, xlv, 249. Reichel: Deutsch. Ztschr. f. Chir., 1892-93, xxxv, 551. Nothnagel: Beitr. z. Physiol. u. Path. d. Darnes, 1884, p. 29. Quirot: Thèse de Paris, 1909, p. 25. These men have all remarked on the contracted, empty state of the bowel above the obstruction, and the signs of anastalsis a long distance above. Shimodaira: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1910, xxii, 229, and Maleyx, Thèse de Paris, 1912, have written monographs on the well-known distention and ulceration of the cecum with obstruction in the lower colon.

64. Kirstein: Deutsch. med. Wchnschr., 1889, xv, 1001, thinks acute ileus is not the result of closure of the bowel but of its maltreatment. When he cut the ileum across and sewed it up, the food went on down the intestine and packed against the obstruction. The dogs did not vomit, and finally died of starvation. When, however, he pinched the bowel with an elastic ligature, the food was turned back in the duodenum and the dogs were very sick. Treves says

The commonest lesion near the ileocecal valve is appendicitis; and this, as is well known, can generally be diagnosed by the presence of marked ileac stasis. I believe chronic appendicitis disturbs digestion mainly through its effect in raising the tone of the terminal ileum; and operations give relief only as they remove all sources of irritation from this region.⁶⁵ A rise of tone here has a retarding effect that extends upward even to the stomach, which is frequently dilated and atonic. The duodenum is often dilated, and material stagnates either in the "cap" or just above the duodenojejunal junction.⁶⁶ That this is not due to kinks or adhesions is shown by the fact that the swallowing of a mouthful of solid food generally causes this bismuth to whisk over into the jejunum. This region has also been found normal at subsequent operation in several cases. I have seen active reverse peristalsis in the duodenum due to the ileac stasis alone.

Back pressure from the intestine must be looked for in all cases of gastric atony or deficient emptying when no lesion can be found near the pylorus. Several writers have emphasized the importance of gastric dilatation as an early sign of intestinal obstruction. It can be detected with the stomach tube and Roentgen

that in acute intussusception, violent symptoms of obstruction may run their course without there having been produced any actual occlusion of the bowel. He believes the symptoms are due to what he calls peritonism, *Intestinal Obstruction*, New York, 1902, p. 2. These mechanical factors should not be lost sight of, although the recent work on intestinal obstruction shows the problem to be quite complicated.

65. Barclay: *Brit. Jour. Surg.*, 1915, ii, 645. According to the theory, we would expect to find hypermotility in the colon with appendicitis. Deaver says diarrhea is present in about 13 per cent. of cases. It is met with more often in children. *Appendicitis*, Philadelphia, 1905, p. 226 and 244. That it is not seen more frequently is due probably to the powerful repelling effect from the lower colon.

66. I have observed these things many times. See articles by Borgbjarg: *Verzögerung der Magenentleerung bei Darmkrankheiten*, *Arch. Verdauungskr.*, 1911, xvii, 706. Bassler: *THE JOURNAL A. M. A.*, Oct. 24, 1914, p. 1469. Eisen, *Ibid.*, p. 1228. Barclay: *Arch. Roentg.* Ray, 1913, xviii, 234. Paterson and other surgeons have seen at operation marked dilatation of stomach and duodenum in many cases of appendicitis. *Proc. Roy. Soc. Med. London*, 1910, iii, *Surg. Sect.*, p. 197. Of 482 cases with gastric stasis due to ascending influences only twenty-one showed retention after the removal of appendices or the restoration of normal conditions in the bile tract. Smithies: *Am. Jour. Med. Sc.*, 1915, cxlix, 187. Cole says, "I have observed time and time again that if, after the contents of the stomach and 'cap' have remained stationary for some time, the ileum be partly evacuated, a rehabilitation of duodenal peristalsis will be established forthwith and food may be seen . . . passing through the jejunum." *Am. Jour. Med. Sc.*, 1914, cxlviii, 109. Barclay has made similar observations, *Brit. Jour. Surg.*, 1915, ii, 643.

ray some time before the reverse currents are strong enough to cause the typical vomiting.⁶⁷

There is a so-called gastric type of carcinoma of the lower colon in which nausea, vomiting and gastric dilatation are the early symptoms of the ascending influences.⁶⁸ The descending ones show themselves later in tenesmus and protrusion of the rectal mucosa.

CONSTIPATION

In sensitive people and in children, constipation may cause vomiting which will yield promptly to a laxative. These patients may feel stuffed after a few mouthfuls, on account of the reverse influences.⁶⁹ Conditions seem to be somewhat as they are in baseball when the bases are full and the batter has made a hit: there is no use of his running if the runner on third refuses to come "home." The intestine may also be thought of as a railroad under control of a block system. Just as food in the stomach forces material through the ileocecal valve or into the rectum when the tract in between is empty, the stagnation of material in a lower block will hold up the food for several blocks above.

Future experiments must show how much actual reverse waves or ripples have to do with the process. The reverse influences seem to skip over regions in which the waves are catastatic to cause anastalsis above. The direction of peristalsis may change also from time to time in the same stretch of bowel. All these observations are explainable in the light of the theory.

A large part of what is called "biliousness" and "autointoxication" may be due to this tendency to reverse peristalsis when the colon remains filled and active. "Biliousness" derives its name from the appearance of bile in regurgitated material, and this would be more liable to occur when the tone of the

67. Ewald: *Berl. klin. Wchnschr.*, 1907, xlix, 1416. Cataldi: *Polislinico*, Rome, 1914, xxi; abstr., *THE JOURNAL A. M. A.*, Jan. 23, 1915, p. 378.

68. Quirrot: *Thèse de Paris*, 1909. Hellblom and Cannm's experiments are in accord with these clinical observations. When they irritated the colons of cats with croton oil, the stomachs emptied very slowly in spite of active peristalsis; and there was great retardation in the small intestine also. *Am. Jour. Med. Sc.*, 1909, cxxxviii, 520.

69. Hertz found patients could eat little at a time and complained of a sense of fullness when the stomach emptied too fast after a gastro-enterostomy. This disagreeable sensation was less after taking castor oil, apparently because the jejunum emptied faster, *Ann. Surg.*, 1913, lviii, 466.

intestine is higher than that of the stomach. Much of the satisfaction that the "bilious" get from a dose of calomel may be due to the setting of a strong current down the tract again. The symptoms in the "auto-intoxicated" ordinarily clear up within a few minutes after emptying the rectum, and no toxin could be eliminated at that rate.

There seems little doubt that normally the tone of the rectum and sigmoid is higher than that of the cecum and ascending colon.⁷⁰ This tends to protect the anal sphincter and to keep feces out of the ampulla except during those times when the pressure from the upper part of the tract is very great. Material which is forced over into the rectum may even be returned to the transverse colon if defecation is postponed.⁷¹ The commonest cause of constipation is probably an increase of this tone which makes the gradient steeper up to the rectum.⁷² Case has found adhesions irritating the pelvic colon in a number of these cases.⁷³

Hemorrhoids and painful fissures may cause constipation not only on account of the fear of pain at defecation but by raising the tone of the rectum, and thereby keeping the sigmoid and ampulla empty. This holding back is often shown by a large accumulation of gas in the splenic flexure. This is so constant a finding that I have come to regard a large tympanitic Traube's space as almost diagnostic of hemorrhoids.

CONCLUSION

It was natural that when the Roentgen ray first threw light on conditions in the digestive tract, the striking defects, as seen in carcinoma and ulcer, and the marked ptoses, so different from what anatomists had taught us to expect, should have occupied the center of the stage. Soon the more thoughtful radiologists began to realize that it was only occasionally that they could see the lesion, or, more correctly, a cast of the lesion. In the large proportion of cases they could only suspect the presence of some irritant from the altered motility in different parts of the tract. Finding marked ptoses and kinks every day in people with

70. Rost: *Verhandl. d. deutsch. Gesellsch. f. Chir.*, 1912, p. 169.

71. See a very interesting article by Campbell: *Tr. Am. Gynec. Soc.*, 1878, p. 286. Also Schwarz: *München. med. Wehnschr.*, 1912, lix, 2155.

72. Sinker and Holzknicht: *München. med. Wehnschr.*, 1911, lvin, 2536. Keith: *Brit. Jour. Surg.*, 1915, ii, 581.

73. Case: *Arch. Roentg. Ray*, 1915, xix, 380.

good digestions, they began to see that a stomach might empty just as satisfactorily when in the pelvis as when above the navel. As Case says, "with increasing experience, the morphological factors have shrunk in importance, while the problems relating to the functional behavior of the alimentary tract have assumed greater significance."⁷⁴

Some good may be done by this article if it helps at this time to bring about a more physiologic point of view in gastro-enterology; if it induces more men to think of the tract as a unit, and to study it from one end to the other before they make diagnoses, or attempt repairs at any one point. Good men are still reporting statistics on gastric ulcer, six-hour stasis, etc., without making mention of conditions even as far away as the gallbladder. Surgeons are doing gastro-enterostomies on baggy stomachs when the offending appendix should come out instead. How much better it would be to explore the tract carefully before beginning work on some part which, as Mayo says, may be the fire-alarm and not the fire.

In the last two years I have found it a very useful rule to remember that: *an irritating lesion slows the progress of food coming toward it from above and hastens the progress of food that has passed it.* This has helped in analyzing many unusual cases. For instance, a number of signs may point to a duodenal ulcer, yet the usual hypermotility is lacking. Further study may show that this has been neutralized by the back pressure from a chronic appendix.

In such a short paper little can be done except to present views that can be discussed in more detail later. Such papers that may follow can more easily be seen then in their relations to the whole problem. More proof can be adduced for many of the statements made here, and objections can then be taken up.

In closing I wish gratefully to acknowledge my indebtedness to Dr. Cannon of the Department of Physiology at Harvard, to Dr. Pope of the Surgical Research Laboratory at the University of California and to Dr. Whipple of the Hooper Research Foundation who, one after the other, in the last two years, have so generously afforded me the opportunities to work on this problem.

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74. Case: Arch. Reintg. Ray, 1:15, XIX, 375.

IV. DIFFERENCES IN RHYTHMICITY AND TONE IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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Before taking up the differences observed in the stomach, it may be of interest to review briefly the theoretical considerations that led up to the work. When it was seen that the rate of rhythmic contraction in the small intestine varies inversely as the distance from the pylorus (1), the next question to arise was: If this part of the primitive intestinal tube behaves in this way, how about the other parts? Might not the tract have been constructed originally so that the rate would be highest at the pharynx and lowest at the anus? Although this question cannot be answered satisfactorily as yet, there is considerable evidence in favor of such a view. For instance, the rates of contraction in different parts of the colon (of the rabbit and cat) fit quite well into a prolongation of the curve plotted from the rates of the small bowel (2). The rhythm varies (in the rabbit) from 6 to 16 per minute near the cecum to from 3 to 5 per minute near the anus. It is impossible to say much about the esophagus of mammals because, in them, that tube is made up almost entirely of striated muscle, and the smooth fibers, with which we are concerned, appear only in the lower third or fourth. Longitudinal segments from this region near the cardia (in rabbits and cats) showed a high rhythmicity when placed in aerated Ringer's solution. The fastest rate seen in the cat was 14 per minute; in the rabbit it was sometimes as high as 19 per minute. It should be noted that this is a higher rate than that ever seen in the duodenal segments (15 to 17.5 per minute).

We can compare the rhythm of different regions of the esophagus only in those lower animals in which the tube is made up entirely of smooth muscle. This is the case in the frog. Stiles found in the esophagus of this animal that the rhythmic activity is more marked and

regular than in any other part of the digestive tract; also, that the rate of contraction (in the esophagus) varies inversely as the distance from the pharyngeal end (3). I have confirmed these findings in a number of frogs; and, although my tracings are not so regular as Stiles', they show the difference in rate very clearly. I have found similar differences in longitudinal segments from the esophagus of a small grass snake (species unknown). The contractions in the intestinal segments from the frog unfortunately were so irregular that I could not establish a further gradation of rhythm from the end of the esophagus down to the cloaca. The only thing that can be said is that the esophageal rates were generally faster than those of any part of the bowel.

Even if further work on such animals should show definitely a gradation of the rhythmic activity from pharynx to anus, we would still have to explain the slow rhythm of the gastric waves in mammals: from 3 to 4 per minute in the rabbit, dog and man, and from 4 to 6 per minute in the cat. A possible way out of this difficulty was suggested to me by the literature on another muscular tube—the heart. Gaskell taught us to view that organ as an elaboration of a simple tube which had become twisted on itself, and had bulged in places. There the muscle became specialized that it might contract and empty the cavities more quickly. "The development of this nearer approach to striated muscle is made at the expense of the original rhythmical power" (4).

THE PRIMITIVE TUBE

A glance at figures 1 to 34 in Oppel's Comparative Histology (5), or at plates 18 to 33 in Huntington's book (6), will show how the stomach also has been evolved from a simple tube, first by an enlargement, secondly, by a bending of the pylorus towards the cardia, and thirdly, by the addition of cecal pouches. The stomach of the eel consists almost entirely of such a pouch, which has grown from the convex side of a bend in the original tube (see fig. 1, *D*). It is very obvious, in such a stomach, that the primitive tube is to be found along the lesser curvature. Even the complicated stomachs of ruminants can be resolved into a series of ceca arranged along the original tube (fig. 1, *E*). That part of the fundus to the left of the cardia in the human stomach represents such a cecum, which, very early in life, grows out from the greater curvature (7). The stomach of a 10 mm. human embryo is made up of three parts: the expanded, conical, lower end of the esophagus, the long tubular antrum, little larger than the adjacent duodenum, and a

small fundus (fig. 1. *F*). The end of the esophagus meets the antrum at the incisura angularis. Later, the fundus grows at the expense of the other two parts, so that, in the adult, the end of the esophagus is represented only by the cardiac antrum and that prolongation along the lesser curvature which forms the gastric canal; while the pyloric antrum makes up a much smaller part of the stomach than it did originally (8).

The "Primitive Tube," accordingly, must be looked for along the lesser curvature. It is suggestive that this part of the stomach is

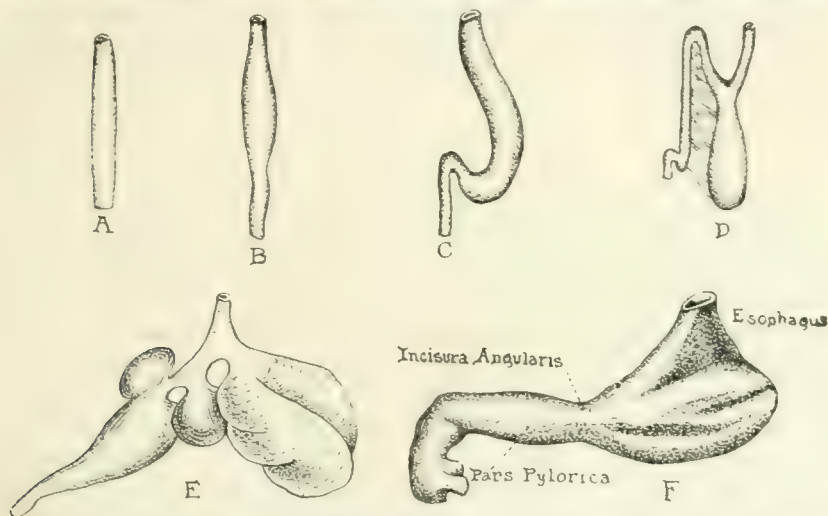


FIG. 1. To show the development of the stomach. *a*, Stomach of the pickerel (Nuhn); *b*, stomach of *Proteus anguineus* (Nuhn); *c*, stomach of *Scincus ocellatus* (Nuhn); *d*, stomach of the eel (Huntington); *e*, scheme of the ruminant compound stomach (Nuhn); *f*, stomach of a 10 mm. human embryo (Lewis).

lined by an epithelium differentiated least of all from that of the intestine. This point has been remarked upon by several men in discussing the mucous membrane of the pyloric antrum. The glands around the cardia are apparently little more than sluggish pyloric glands (9); and "it is almost the rule for the greater part of the mucous membrane along the lesser curvature to be of the pyloric type" (10). A similar arrangement is found in most of the domestic animals, that is, the lesser curvature is lined only by cardiac and pyloric glands (11).

To be sure, we must be careful in comparing conditions in two organs so different in function as are the heart and stomach. One has been

specialized to pump blood rapidly; the other serves largely as a reservoir, a hopper for the bowel, where the waves do more mixing than propelling. Yet they have both been evolved from simple tubes of rhythmic muscle, and it seems to me that the analogies are close enough to make us eager to examine the stomach from a point of view which has done so much to advance our knowledge of the physiology and pathology of the heart. In the stomach, we should expect to find the most rhythmic tissue at the cardia and along the lesser curvature. The least rhythmic tissue might be in the fundus and along the greater curvature. Such differences, if present, might go far to explain the origin and peculiarities of gastric peristalsis. I wish to show now to what a considerable extent these expectations have been fulfilled.

TECHNIC

After some experimenting, good records were obtained from longitudinal strips of muscle from different parts of the stomach of the rabbit, cat, dog, and man. With a razor, parallel cuts 3 to 5 mm. apart were made just to the mucosa. A narrow strip of muscle 2 cm. long was then lifted up, after cutting through the submucosa with a fine pair of scissors. The only place in the rabbit where this was impossible was along the *canalis gastricus* from the cardia to the *incisura angularis*. Here I could find no line of separation, so the muscular strips from this part of the lesser curvature were studied with mucous membrane attached (fig. 2, *A* and *B*). A separation could be made in the cat, dog and man, although it was more difficult in this region than in the rest of the stomach. This close attachment of the mucosa to the muscle brings to mind a similar arrangement of skin and fascia in the palm of the hand, which enables us to grasp things firmly. In the same way, in the stomach, this intimate relation between muscle and mucous membrane may be essential to the formation of the *canalis gastricus*, through which fluids flow along the lesser curvature (12). A localized contraction on the greater curvature might not show at all on the inside of the stomach, as the mucosa there is redundant, and but loosely attached to the muscle.

Separation of the strips was very easy in the pyloric antrum, and the laxity of the submucosa in that region was striking in all the animals studied.

The strips were usually immersed in warm aerated Ringer's solution and studied at once, but they can be kept in the icebox for four or five

days. It is remarkable that daily tracings from the same set of strips showed that they might beat even better on the second or third day than on the first. This seemed to be due to a loss of inhibition, as the strips generally began beating more promptly after immersion; the rhythm often was faster, and the records more regular. A great deal of patience was needed with fresh strips, as many of them did not show activity until they had been in the warm Ringer's solution for an hour or more. Even then, one part of barium chloride to 25,000 of the solution often had to be added before some of them would beat. The temperature of the Ringer's solution was kept between 37° and 38°C. Ordinarily no weight was added to the light heart levers used; it was not found to be necessary.

EXPERIMENTAL DATA

The following conclusions are based upon records from the stomachs of sixteen rabbits, eight cats, nine dogs and one man. This material seemed to be sufficient, as most of the data were in such entire agreement. Longitudinal strips from the two curvatures have been used almost exclusively. Some work was done with pieces cut longitudinally midway between the two curvatures and with circular strips from different regions, but it was soon discontinued, as the only ones that showed much activity were those from the neighborhood of the cardia. The type of contraction obtained in the circular strips corresponded to that of the longitudinal ones from the same region.

The first strips to begin contracting after immersion were those from the upper end of the stomach. In the cat and dog the strip from the lesser curvature next to the cardia (fig. 3, *A*) was first, often showing activity immediately after immersion in the bath. In the rabbit, the strips from the fundus (fig. 2, *D* and *E*) seemed to recover sooner from the trauma of attachment and generally became active shortly before the cardiac strips did. Strips from the greater curvature and from the antrum (particularly in the rabbit) often took an hour or two to get started, and even then some did not contract well. Thus, out of eleven strips from the rabbit's antrum, only two showed rhythmic activity.

It should be emphasized that the only strip that could be counted upon in every stomach to give regular, typical tracings was that cut from the lesser curvature near the cardia (*A*, figs. 2 and 3). *This region showed the greatest tendency to rhythmic contraction of any part of the stomach.*

THE DIFFERENT TYPES OF CURVES

The curves traced by strips from certain regions of the stomach were so characteristic that there was little need for labelling some of them. This might be said particularly about the records of the strip from the lesser curvature of the rabbit near the cardia. The individual

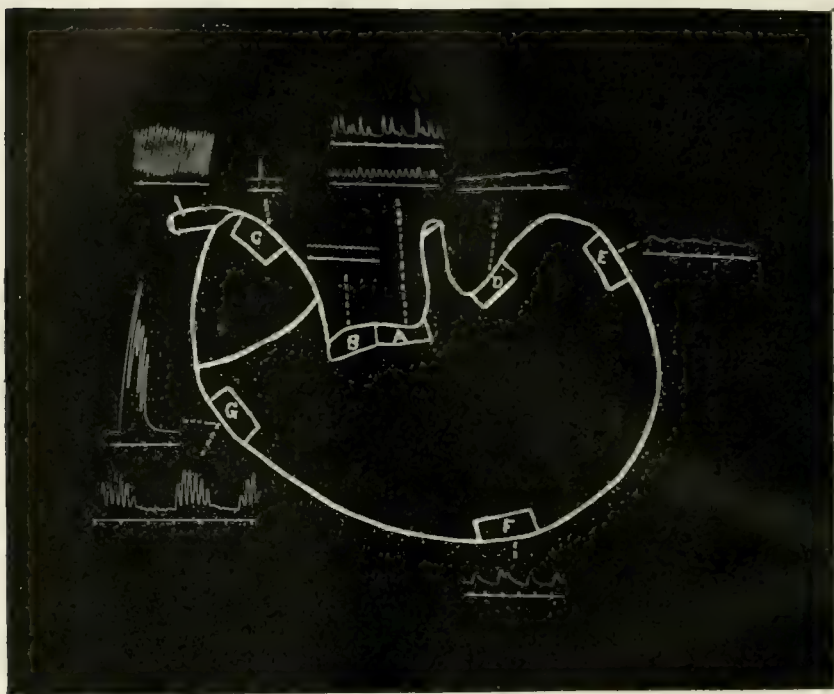


FIG. 2. Diagram of the rabbit's stomach showing the location of the principal strips studied, together with specimen tracings from the different regions. At A and G two characteristic types of tracing are shown. The time tracing represents 30 second intervals. A short strip of duodenal tracing is inserted for comparison.

contractions of this strip could generally be recognized by the almost vertical rise and the sharp peak caused by the immediate relaxation (see figs. 2 and 4). This type of curve often shaded into another very regular form, particularly when the rate became faster, or after the addition of 1:25,000 PaCl_2 (fig. 4). In the cat and dog, the curves from this region could be recognized not only by the sharper apices to the

contractions and the more rapid rate, but often on account of the peculiar tonus waves depicted in figure 5.

Strips from the lesser curvature (*B*, figs. 2 and 3) beat with a very small amplitude in all the animals. In the rabbit, the waves could sometimes be made out only by using a hand lens. Many strips did not beat at all. The possible reasons for this will be taken up later.

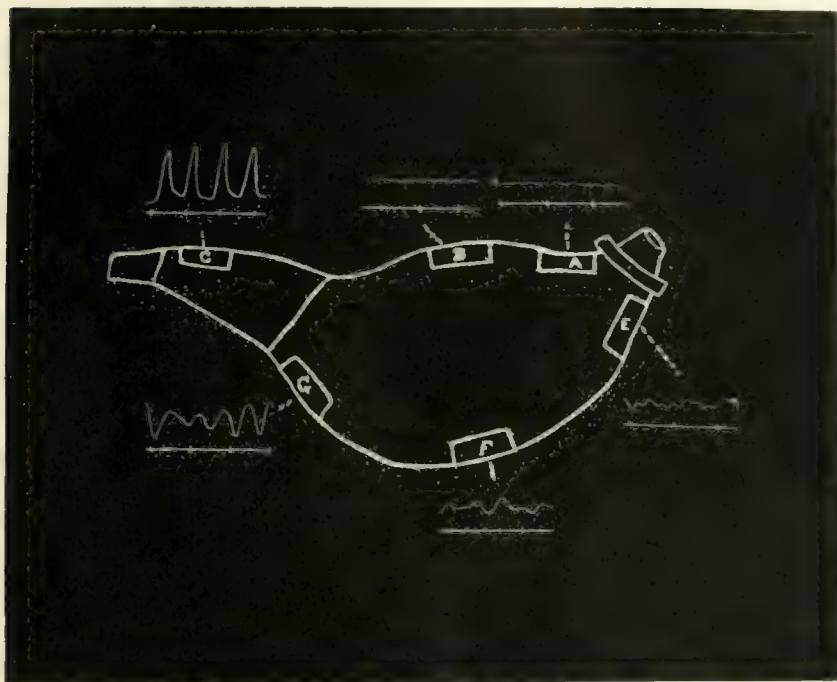


FIG. 3. A diagram of the cat's stomach to show the location of the principal strips studied and the types of tracing peculiar to the different regions. This diagram will serve also for the location of strips in the dog's stomach. The time tracing represents 30 second intervals.

In the rabbit, strips next to the cardia on the side towards the fundus (fig. 2, *D*) gave tracings very different from those just described for strip *A* on the side of the lesser curvature. They were characterized by marked irregularities of tone, rhythm and amplitude. Sections from the rest of the fundus behaved in much the same way. In the cat and dog there was less difference in the behavior of the strips on the two curvatures next to the cardia (fig. 3, *A* and *E*). This is to

be expected when we remember that their stomachs have almost no fundus and that they are simpler and less differentiated from the original tube.

Strips from the middle region of the greater curvature in all the animals (*F*, figs. 2 and 3) varied a good deal in their reactions. Some did not beat at all, others gave fair tracings, while a few were quite regular. The best curves in the rabbit were seen after the tone had been raised by barium chloride; and particularly in strips which had been on ice from 24 to 48 hours. They never resembled the typical ones from the cardia however. Ordinarily, the waves were large, rounded and uneven. They were even larger and more rounded in the cat and dog.

Strips from the greater curvature near the antrum in the rabbit's stomach gave peculiar curves characterized by regularly recurring

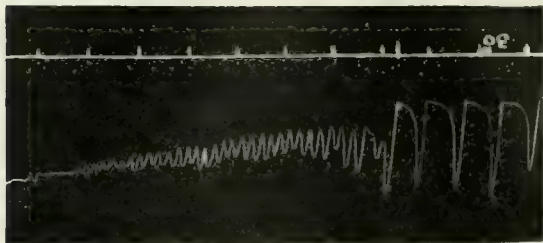


FIG. 4. Tracing from a strip from the lesser curvature near the cardia showing the change in rhythm after adding 1: 25,000 BaCl_2 .

groups of waves with a wide amplitude. Sometimes the muscle strip would shorten to less than half its original length. Two such curves are shown at *G*, figure 2. Particularly after the addition of a little barium to the solution, this type of curve often shaded

into another, as regular and even as a duodenal tracing. This very regular curve with rapid rhythm was seen in some strips twenty-four hours old from the same region of the cat's stomach. Ordinarily in the cat and dog these strips reacted very much like those from the middle of the greater curvature.

The type of contraction in the strips from the antrum pylori (*C*, figs. 2 and 3) was very characteristic in all the animals studied. It made no difference from what part of the antrum they were taken. The curves showed a very even base line, upon which were superimposed at regular intervals high symmetrical peaks. These are well shown in figure 6, the middle record. It was very typical even in the frog, where the great amplitude of contraction in this region was well brought out. On such tracings the antral peaks were often four times as high as the cardiac ones, in spite of the fact that the antral strip might

be only a fourth as long as the strip from the much wider cardiac end of the stomach. This difference is well illustrated by fig. 10 in an article by Woodworth (13)

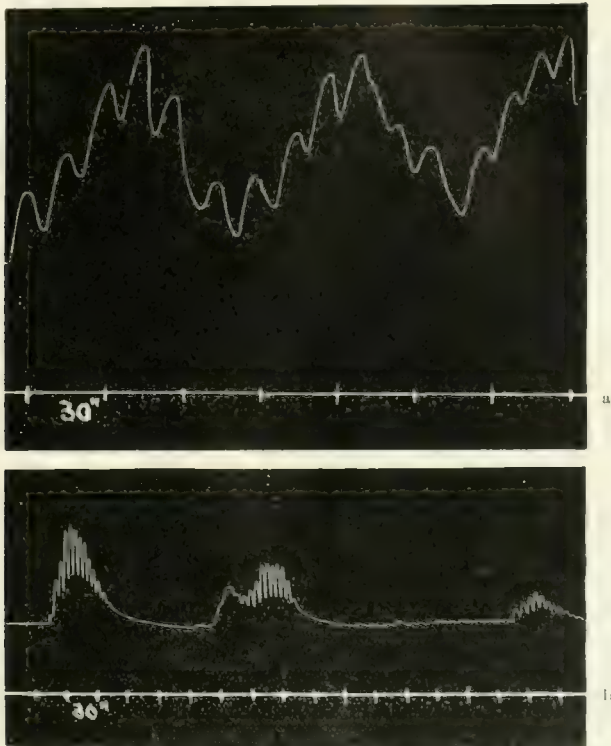


FIG. 5. *a*, Tonus waves in a record from the strip on the lesser curvature next to the cardia of a cat's stomach. *b*, From the same region in a dog's stomach.

DIFFERENCES IN THE RATE OF CONTRACTIONS

Speaking roughly, the rate varied as the distance from the cardia. The fastest rates in all the animals were observed in the tracings from strip A on the lesser curvature. In the cat, this strip contracted from 4 to 8 times per minute. In the rabbit and dog there were two or three different types of curve with different rates. Often when the strips first began to beat after immersion, the rate would be from 2 to 5 per minute. After awhile the contractions would become smaller and more

frequent, and the rate would change to from 9 to 14 per minute, usually about 11. Three strips beat 20 times per minute for short intervals. The rate in the dog was usually from 8 to 13 per minute, but in a few animals it was from 4 to 7 per minute. At the other end of the stomach, in the antrum, the rates varied from 1 to 4 per minute in all the animals. The rates of the other strips ranged between these two extremes: usually from 4 to 9 per minute. One exception must be made in regard to the strip on the greater curvature next to the antrum in the rabbit (*G*, fig. 2). Here the rate was often from 9 to 12 per minute. The possible significance of this will be discussed later.

DIFFERENCES IN TONE

Differences in tone were observed while studying the strips. When the cuts were made through the muscle on the lesser curvature, the edges pulled apart farther than they did on the greater curvature. Strip A on the lesser curvature was always much smaller than the hole from which it was removed, but strips E and F on the greater curvature might be even larger than the hole, if care were not taken to avoid all traction. Strips E and F would often lie flat when put into Ringer's solution, but strips A, B and G generally curled up tightly.

A high tone on the lesser curvature might have something to do with the poor amplitude of contraction in the strips from this region. I have commented elsewhere (14) upon the fact, so often observed with smooth muscle, that as the tone rises, the amplitude of contraction falls until rhythmic activity may cease entirely. The larger amplitude of contraction seen in the strips from the greater curvature agrees perfectly with the supposition that the tone is low in that region. The great amplitude of contraction in the strips from the antrum and pre-antrum is probably due to other factors, as the tone seemed to be high. The fibers of the muscle might be longer or more nearly parallel in this region. Such histological differences have been found to explain differences in the reactions of the frog's sartorius and gastrocnemius (15). It is suggestive that McGill (16) has noticed histologically a tendency to almost total contraction of muscle fibers in the pyloric ring.

Tone, unfortunately, is a vague and often misused term. Sherrington has shown recently (17), that many of the phenomena attributed to it are really what he calls "Postural" changes, that is, there is an adjustment of the contractile length of the muscles without necessarily

an alteration of tension. Such adjustments must take place constantly in the fundus of the stomach so that it can maintain a steady even pressure on the material that is being fed into the rhythmically contracting pyloric mill. This may explain the marked tendency of excised strips from the rabbit's fundus to contract down to about one-half of their original lengths after they have been in the warm Ringer's solution for from 30 to 60 minutes. After this, they seldom relaxed or showed much rhythmic activity. It is interesting to note the resemblance of the tonus waves in a strip from the neighborhood of the dog's cardia (fig. 5b) to those observed by Fano, Porter (18) and others in the auricles of the toad and terrapin. Such changes have been noticed near the cardia in the intact stomach also (19).

THE HUMAN STOMACH

The kindness of Doctors Baxter and Brill enabled me to get the stomach of a man within a half hour after death from nephritis. Strips from this stomach reacted very much like those already studied. Figure 7a shows the small, regular and rapid rhythm in the cardiac strip from the lesser curvature. The rate varied between 5 and 12 per minute. The next piece on the lesser curvature showed a few contractions, only after barium was added. The strips from the preantrum on the lesser curvature contracted very much like those from the greater curvature in some

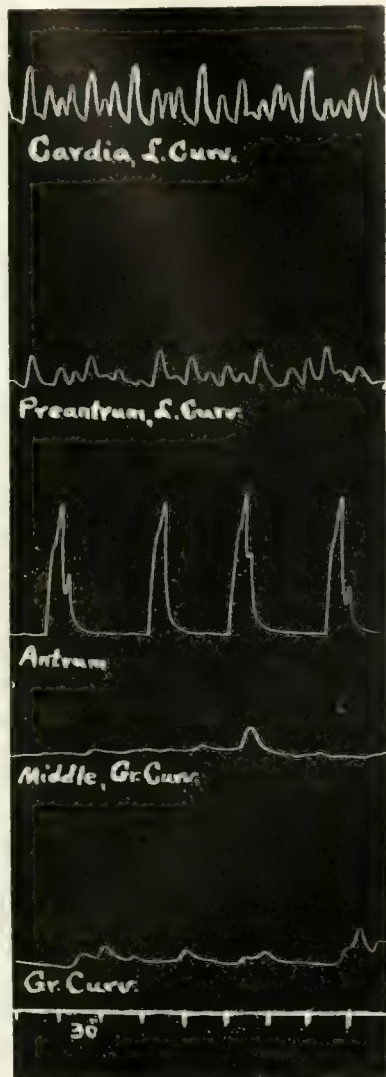


FIG. 6. Records from five strips from different parts of the dog's stomach. Shows typical contractions from the pyloric antrum.

cats. The amplitude was large, the rhythm slow and irregular. The strip from the antrum pylori on the greater curvature showed the usual type of curve for that region. Strips from the greater curvature showed less rhythmicity than did those from the lesser curvature.

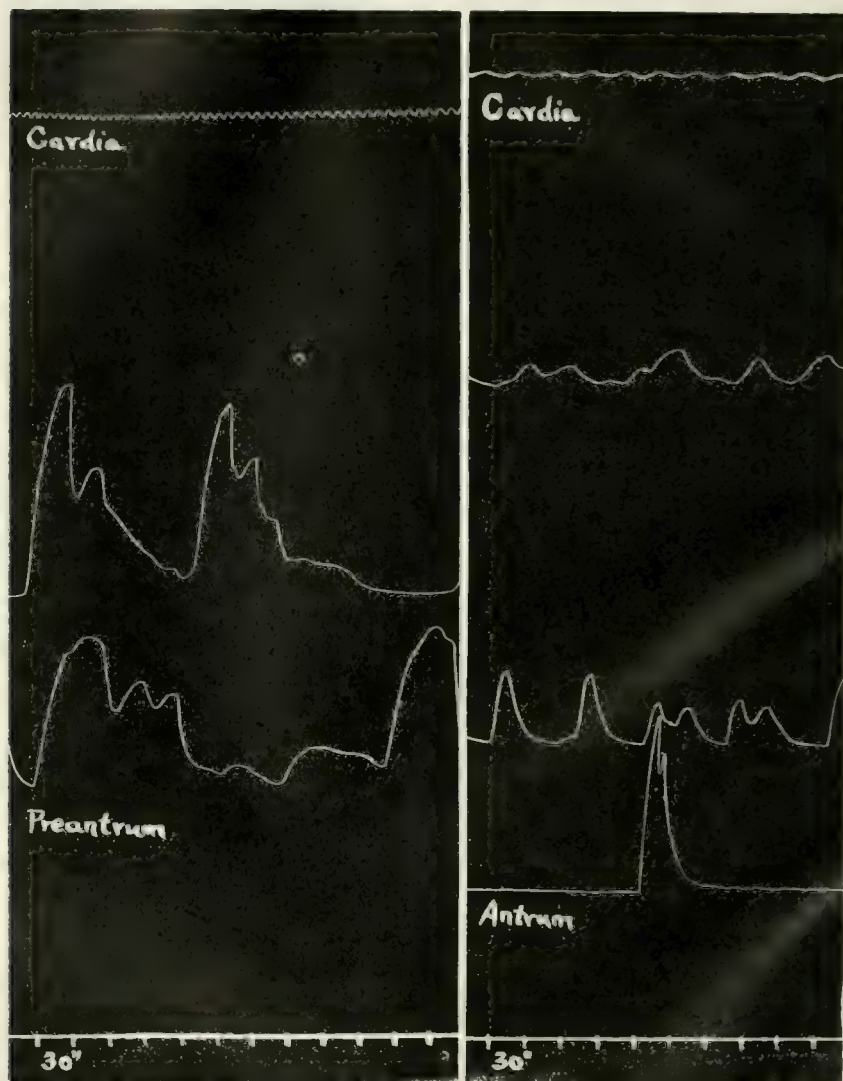


FIG. 7. *a*, Records from four strips from different parts of the lesser curvature of a human stomach. *b*, Four strips from the greater curvature.

DISCUSSION

As was expected, marked differences have been found in the behavior and in the rhythmicity of the strips. Some of these peculiarities, such as the high rhythmicity of strip A, the grading of the rhythm downwards towards the pylorus, and the differences in tone on the two curvatures are explainable on the basis of the theory that gave rise to the work. Other features, such as the low rhythmicity of strips from the middle of the lesser curvature, the promptness with which strips from the rabbit's fundus began contracting after immersion, and the high rhythmicity of the preantral strip on the greater curvature are rather against the view that the rhythmicity should vary inversely as the distance, spacially or embryologically, from the primitive tube.

There are probably other modifying factors present, such as those tending to fit the muscle in the different regions to the different types of work that have to be done. Such a factor might account for the marked differences between the behavior of strips from the pyloric antrum and from the body of the stomach. A remnant of the original tube itself might lose much of its rhythmicity if that function should interfere with the work to be done. This might be one explanation for the poor records obtained from strips from the *canalis gastricus* along the lesser curvature of the rabbit's stomach. The high rhythmicity of strip G on the greater curvature in the rabbit is not so easily explained. Auer noticed on the intact stomach that after reflex inhibition of the movements, they always returned first in the preantral ring, and he concluded that this was clearly the most rhythmic section (26). I believe, however, that it is exceeded in this regard by the cardia. These problems cannot be settled on the basis of differences in rhythmicity alone. More light must be obtained by studying the irritability, latent period, conductivity, etc., in the different regions. For instance, as will be seen in the next paper, a comparison of the latent periods in different parts of the stomach seems to support the original theory even more than has the study of differences in rhythmicity.

It took many experiments and years of discussion to establish the fact that the stomach can perform its functions quite satisfactorily and normally after section of all extrinsic nerves (21). The observations presented in this paper show now that local peculiarities of tone and rhythmicity may have much to do with directing and modifying the peristaltic wave as it travels over the stomach. A glance at one of

Groedel's (22) illustrations made up of the superimposed outlines of a dozen serial radiographs of the same human stomach will show how little the lesser curvature, as far as the *incisura angularis*, is affected by the peristaltic wave. Appearing at a variable distance from the fundus, the waves seem to travel almost entirely along the greater curvature, getting deeper as they approach the antrum. At that point, their character changes markedly; they involve the whole circumference of the stomach and are so deep that they sometimes meet in the center.

It seems to me that these local differences in the peristaltic wave correspond perfectly to the regional peculiarities of tone, rhythm and amplitude of the tissue through which it must pass. As in the heart, so here, the waves probably have their origin in the most highly rhythmic area. Similarly, again, the gradation of rhythmicity from cardia to pylorus may have much to do with maintaining the downward course of the waves.

Conduction must be very different in the two organs, as, in the stomach, the waves keep traveling quite normally after several encircling cuts have been made down to the mucosa (23). Moreover, the pyloric portion of the stomach in dogs continues to functionate normally even after complete separation from the rest of the organ (24). There is also little interference with peristalsis in the human stomach after excising the middle portion for carcinoma (25).

Rather against the view that the waves originate near the cardia is the common observation that they seem to appear now here, now there on the greater curvature. As Cannon says, "the pulsatile source of the gastric waves has no fixed seat" (26). His work showed that a wave is likely to appear at the spot where a certain balance is struck between the tone of the muscle and the internal tension. My records from the intact intestine showed clearly that a peristaltic rush which apparently had begun in the lower ileum had really come as an unnoticed ripple, all the way from the duodenum (27). I believe that the same thing may take place in the stomach, and that ripples sent out from the cardia may deepen into large waves at the place where the conditions defined by Doctor Cannon are right. Perhaps we could see these ripples if we had better means of detecting what is going on. As Groedel (28) says, anyone watching peristalsis in the human stomach would say that the lesser curvature did not participate at all, yet good serial plates always showed waves corresponding to those on the greater curvature. Dietlen (29) has shown also that with the patient lying

down, so that the fundus is filled, small but definite waves can be seen near the cardia.

Another question that arises is, why should the rates of the strips in the rabbit and dog be so much higher than that of the intact stomach. Only in the cat do they correspond at all. It is different in the intestine, where the rates of the intact bowel and of the excised segments agree quite closely. In the rabbit it may be that the slower, more powerful contractions that were seen in many of the tracings from the cardiac strip are the ones that initiate the peristaltic waves of the stomach. The faster rates may indicate a reserve, of which the cardia has the greatest amount. It does not seem likely that the normal slow rate is due to depressor effects from the vagus as peristalsis is not quickened after double vagotomy (30). More probably the longer intervals between beats are needed for adequate rest and recovery, so that the muscle can maintain a constant level of efficiency. For the same reason, the medusae pulsate normally at only about one-seventh the rate that they are capable of maintaining under certain conditions (31).

ANATOMICAL DIFFERENCES

It is hoped that this work may induce histologists to seek for regional differences in the nervous and muscular tissues of the stomach, and to study more closely the region about the cardia and the lesser curvature. Openchowski years ago claimed that the automatism of the cardia is due to groups of peculiar ganglion cells under the serosa (32). These cells were like those found in the heart. They were distinct from Auerbach's plexus; and when they were stripped off, automatic movements ceased. This, of course, might have been due to the trauma. He found similar groups of cells near the pylorus, but there were very few in the body of the stomach. Schütz also has described such ganglia grouped about the cardia and pylorus (33). Those in the pyloric portion and fundus had connective tissue capsules. Near the cardia the cells were not in the muscle layers, as they were elsewhere in the stomach, but were in the connective tissue between the layers.

Keith (34), who has recently done some very interesting work on this problem, found the myenteric plexus well developed only in the pyloric division and along the lesser curvature. There was no localized increase or development at the point where gastric movements ordinarily seem to begin. There was, however, a "distinct modification of the musculature and myenteric plexus just distal to the ring which

marks the cessation of the esophageal epithelium and the commencement of the gastric lining. At that site there was a definite development of neuro-muscular junctional tissue—just such an area as might serve as a nodal center for the stomach.” In this region in the echidna he found tissue similar to that seen in the sino-auricular node of the same animal. He believes the contractions of the stomach are there initiated. Thus, reasoning along the same lines but using different methods, Doctor Keith and I have arrived, independently, at the same conclusion.

Other differences will probably be found in the muscle itself. It is well known that there are marked differences in irritability, latent period and form of the contraction-curve between the pale and red voluntary muscles in the same frog or rabbit, between the flexors and extensors, between the abductor and adductor of the crab's pincers, or between the wing and leg muscles of an insect. Histological differences have also been found corresponding to the functional ones. The proportion between the sarcoplasm and the fibrils varies markedly in different muscles; and even in the same muscle there may be fine and coarse fibers with different degrees of irritability, so that weak and strong stimuli produce different effects. After reading the articles of Ranvier (35), Rollett (36), Grützner (37), and particularly that of Paukul (38), and seeing how remarkably striated muscles vary, not only throughout the animal kingdom but in the individual body, it seems to me unreasonable to expect that smooth muscle should have fixed properties and structure. Marked differences in the physiological properties of bits of smooth muscle from different organs are well known, but I can find very little about histological differences. McGill is about the only one who seems to have observed such details. She found in some parts of the digestive tract a persistence of the embryonic condition as shown by the distinct syncytial arrangement of the muscle fibers with both end and side anastomoses (39). Unfortunately, Doctor McGill had no reason then to note just where those bodies of embryonic tissue were found.

SUMMARY

The evidence presented suggests that the gastro-intestinal tube may originally have been constructed so that the rhythmicity of any one segment varied inversely as the distance from the pharynx.

It is proposed to study the stomach from the point of view that it has been evolved from a primitive tube much as the heart has been en-

larged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature of the stomach from the cardia to the pyloric antrum.

Excised strips of muscle from the cardiac end, and particularly that one on the lesser curvature next to the cardia, show the strongest tendency to rhythmic contraction.

Different types of tracings are peculiar to the strips from different regions of the stomach. Speaking roughly, the rate of contraction varies inversely as the distance from the cardia. The tone seems to be higher on the lesser than on the greater curvature.

Strips from the human stomach behave very similarly to those obtained from the rabbit, cat and dog.

The differences observed in the strips probably determine the direction and local peculiarities of the peristaltic wave as it sweeps over the stomach.

REFERENCES

- (1) ALVAREZ: *This Journal*, 1915, xxxvii, 266.
- (2) ALVAREZ: *This Journal*, 1914, xxxv, 177.
- (3) STILES: *This Journal*, 1901, v, 338.
- (4) GASKELL: Schäfer's *Textb. of Physiol.*, London, 1900, ii, 177.
- (5) OPPEL: *Lehrb. d. vergl. mikroskop. Anat.*, 1896, i, 8.
- (6) HUNTINGTON: *Anat. of the human peritoneum and abdominal cavity*, Phila., 1903.
- (7) KEITH AND JONES: *Journ. Anat. and Physiol.*, 1902, xxxvi, 34.
- (8) LEWIS: *Amer. Journ. Anat.*, 1912, xiii, 500.
- (9) BENSLEY: *Amer. Journ. Anat.*, 1902, ii, 122, 128, 133.
- (10) LANSDOWN AND WILLIAMSON: *Brit. Journ. Surgery*, 1914, ii, 308.
- (11) HAANE: *Arch. f. Anat. u. Physiol.*, *Anat. Abth.* 1905, 1; also BENSLEY (9) 136, et seq.
- (12) JEFFERSON: *Journ. Anat. and Physiol.*, 1915, xlix, 165.
- (13) WOODWORTH: *This Journal*, 1900, iii, 37.
- (14) ALVAREZ: *This Journal*, 1914, xxxv, 188.
- (15) STEWART: *Manual of Physiol.*, 1900, 562; Biedermann: *Electro-Physiol.*, (Engl. Transl.) London, 1896, i, 62.
- (16) MCGILL: *Amer. Journ. Anat.*, 1909, ix, 528.
- (17) SHERRINGTON: *Brain*, 1915, xxxviii, 222.
- (18) FANO: Quoted from Luciani, *Human Physiol.*, (Engl. Transl.) 1911, i, 319.
PORTER: *This Journal*, 1905, xv, 1.
- (19) DUCCESCHI: *Arch. per le Sci. Med.*, 1897, xxi, 134, 137; KIRSCHNER AND MANGOLD: *Mitth. a. d. Grenzgeb. d. Med. u. Chir.*, 1911, xxiii, 477.
- (20) AUER: *This Journal*, 1908, xxiii, 172.
- (21) CANNON: *This Journal*, 1906, xvii, 429.

- (22) GROEDEL: Die Magenbewegung, Hamburg, 1912, figs. 80, 83, 310.
- (23) CANNON: This Journal, 1911, xxix, 258.
- (24) KIRSCHNER AND MANGOLD: Mitth. a. d. Grenzgeb. d. Med. u. Chir., 1 11, xxiii, 446.
- (25) FAULHABER AND V. REDWITZ: Med. Klin., 1914, x, 680.
- (26) CANNON: This Journal, 1911, xxix, 257.
- (27) ALVAREZ: This Journal, 1915, xxxvii, fig. 3, p. 273.
- (28) GROEDEL: Die Magenbewegung, 1912, 68.
- (29) DIETLEN: Ergebnisse d. Physiol., 1913, xiii, 87.
- (30) CANNON: This Journal, 1906, xvii, 432.
- (31) MAYER: This Journal, 1916, xxxix, 379.
- (32) OPENCHOWSKI: Deutsch. med. Wochenschr., 1889, xv, 717; Zentralbl. f. Physiol., 1889, iii, 2.
- (33) SCHÜTZ: Archiv. f. Verdauungskr., 1908, xiv, 242.
- (34) KEITH: Lancet, 1915, ii, 371.
- (35) RANVIER: Compt. Rend. Acad. d. sc. Par., 1873, lxxvii, 1030.
- (36) ROLLETT: Sitzungsber. d. Kaiserl. Akad. d. Wissensch. in Wien, 1875, lxxii, 349.
- (37) GRÜTZNER: Schmidt's Jahrb., 1883, cc, 119.
- (38) PAUKUL: Arch. f. Anat. u. Physiol., physiol. Abth., 1904, 100.
- (39) MCGILL: Amer. Journ. Anat., 1909, ix, 508 and 523.

V. DIFFERENCES IN IRRITABILITY AND LATENT PERIOD IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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I have proposed in a recent paper to study the stomach from the point of view that it has been evolved from a simple tube much as the heart has been enlarged and specialized (1). Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of the primitive intestinal tube along the lesser curvature. These theoretical considerations led to the discovery that strips of muscle from different parts of the stomach wall vary markedly in their tendencies to rhythmic activity, in the rates of contraction, and in the forms of the curves. The most rhythmic region was found on the lesser curvature next to the cardia. The rate of contraction was generally faster and the rhythm more regular in the strips from the lesser curvature than in those from the greater curvature.

Work was done next to see whether differences could be found in irritability and latent period in different parts of the stomach wall, associated with the differences in contractility and based perhaps upon the same developmental peculiarities. Very little could be found in the literature bearing upon the subject. Meltzer (2) observed in dogs and rabbits that faradic stimulation of the outer surface of the stomach in situ gave different results in different regions. The fundus was quite unresponsive. Contractility seemed to improve as he approached the pylorus. Stimulation from the inside of the stomach had no effect except with the strongest currents. A local contraction of the small intestine could be obtained with a much weaker current than that needed for the stomach.

Lüderitz (3) hoped to explain the origin of gastric peristalsis by similar studies. Using mechanical, chemical and electrical stimuli, he noticed that the contraction often appeared cardially to the place stimulated. He thought there was a greater irritability near the lesser

curvature than elsewhere. The reactions were very different in the pyloric antrum. When the stimulus was applied near the pylorus, there was a uniform contraction of the whole distal region. In dogs the fundus was quite insensitive to faradism.

Duceschi (4) studied the irritability of different parts of the interior of the dog's stomach, using balloons with a device designed to apply the stimulus to the region distended. In the body of the stomach, the effects of mechanical stimulation were weak and slow to appear. He thought the reactions were quicker and more energetic at the pylorus than at the cardia. With the faradic current, the cardia proved to be the most irritable region, and its latent period was shortest. Such stimulation at the cardia sometimes caused inhibition when considerable activity was present. The fundus reacted very similarly but was much less irritable. The response of the pyloric antrum was even less than that of the fundus.

Weak acids had a more pronounced stimulating effect on the cardia than on the fundus. In the pyloric antrum, the effect was generally reversed, the active movements being inhibited. The presence of the balloon in the antrum almost always gave rise to active peristalsis. This was not the case in the fundus. When the antrum was contracting rhythmically, it was quite refractory to outside stimuli. This refractoriness of the active antrum was noticed by Rogers and Hardt (5) while studying the means of inhibiting hunger contractions. Carlson (6) has remarked that the movements of digestion are mainly in the pyloric part; the hunger contractions in the fundus. "Either these two regions of the stomach react differently to local stimulation of the gastric mucosa or else the nervous mechanisms concerned . . . are different." May (7) found the inhibitory effect of vagus stimulation more pronounced on the cardiac than upon the pyloric end of the stomach.

A very suggestive paper is that of Barbera (8) who found that when the stomach of a frog was stimulated at any point by weak faradic shocks, the contraction appeared first at the cardia. By inserting a small manometer in the cardia, and by resting heart levers on different parts of the stomach, he found that this was due to a shorter latent period at the cardia. With the same strength of current a rise in the tube appeared 8.4 seconds after stimulating the cardia; 10.2 seconds after stimulating the middle region and 13.5 seconds after stimulating near the pylorus. In only one case, while stimulating the pyloric region, that part contracted first and then the cardia, followed by the

middle. Ordinarily, stimulation anywhere on the stomach was picked up first at the cardia, and this insured the downward course of the peristaltic wave.

RESULT OF EXPERIMENTS

This work of Barbera on the frog seemed to me so important and fundamental that I have repeated it. Difficulties with the manometer method caused me to study the stomach *in situ* in pithed frogs, noting the time elapsed between stimulation and contraction by counting the seconds ticked off by a pendulum. The current was obtained from a Harvard Apparatus Company's inductorium (9) with one dry cell. Platinum electrodes were used, 3 mm. apart. The current was just perceptible to the tongue with the secondary coil at 12.5 cm. Stimulation was kept up for ten seconds, or until a contraction appeared. Care was taken to keep the preparation moist. The work was done in the months of September and October.

Although my results in ten frogs were not so uniform as those of Barbera, the cardia certainly gave by far the quickest reactions. There was generally less difference between the latent periods of the middle and pyloric regions than between those of cardia and middle region, and on four occasions the antrum reacted even more quickly than did the middle. This might have been caused by temporary changes in irritability due to previous stimulation or to the passage of spontaneous waves. The differences in latent period were brought out best by the weakest currents that would take effect in the antrum. Still weaker stimuli showed that the cardia was the most irritable part of the stomach, as it generally responded to currents that had no effect on the middle or on the antrum. Stimulation of the cardia was also more likely to cause a strong contraction of the whole stomach than was stimulation of the antrum. Frequently irritation of the pyloric region brought about contraction at the cardia and in the duodenum before any effect was observed at the point stimulated.

EXPERIMENTAL OBSERVATIONS ON THE STOMACHS OF RABBITS, CATS AND DOGS

A similar technic was followed with the stomachs of rabbits, cats and dogs. The rabbits were killed with a blow on the head; the other animals by etherization and bleeding. The stomach was removed quickly and placed in a shallow dish containing Ringer's solution at

38°C. The upper surface, while being tested, was kept moist by pouring on more warm Ringer's solution. Unfortunately, some cooling could not be avoided. The latent period in almost all of the experiments was determined visually, as has been described. Graphic records were obtained from a few stomachs, enough to show that the simpler technic was sufficiently accurate. These stomachs were suspended vertically and the region to be tested was picked up by two tiny serrefines; the lower one attached to a rigid support, the other to the writing lever. Such methods could not be used regularly on account of the time that would be consumed in making the fifty or more observations required on each stomach. It was feared that during this time there might be such changes in the irritability that no comparison could be made between the observations taken first and those taken last.

The results obtained on any single stomach would have served to illustrate what I wish to show, but it seemed best to average the observations made in the different regions. Figure 1 shows the location of these regions on the anterior surface of the rabbit's stomach, together with the average latent periods for faradic and galvanic stimulation obtained from 10 animals. Figure 2 gives similar data from the stomachs of 8 cats. The faradic current was obtained from the same apparatus that was used on the frog. The secondary coil was kept at 8 cm. in all the experiments. The galvanic current was just strong enough, 1.7 m.a., to duplicate the faradic effects in the cardiac and middle regions. The same platinum electrodes were used.

In the rabbit, the reaction was practically immediate in the esophagus and over the antrum cardiacum. It is well known that striated muscle reaches to the cardia in rabbits and dogs, but the articles consulted did not give the impression that it spreads over the stomach at all. Doctor Whipple kindly looked at some sections from these animals, and found strands extending 2 cm. beyond the cardiac thickening. This complication was not met with in the cat or man, where the lower fourth of the esophagus is free from striated muscle.

As will be seen from figure 1, the latent period was shortest around the cardia and on the lesser curvature as far as the incisura angularis. Beyond that, in the middle of the anterior surface, there is a zone in which the reactions appeared within a second or two. Outside of that again is an area, along the greater curvature, in which reactions were obtained in from one to four seconds in the more irritable stomachs (often from the younger animals). In some of the less irritable stom-

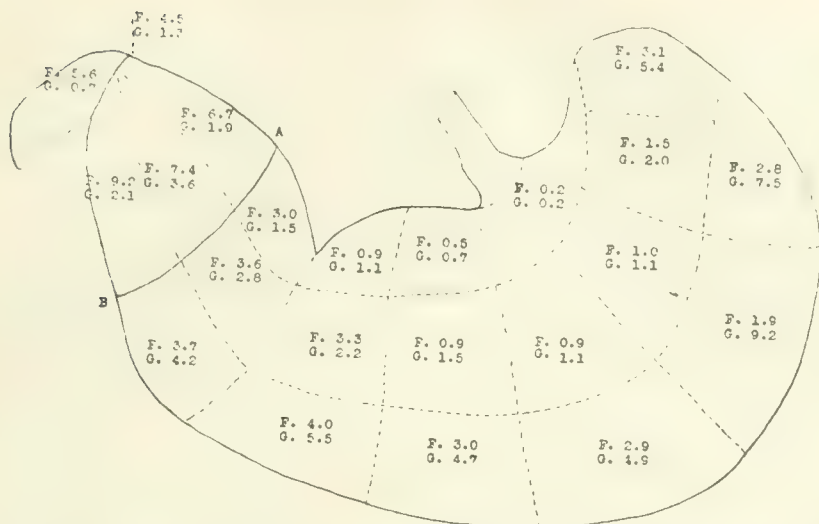


FIG. 1. Anterior surface of the stomach of the rabbit, showing the different regions stimulated, with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach.

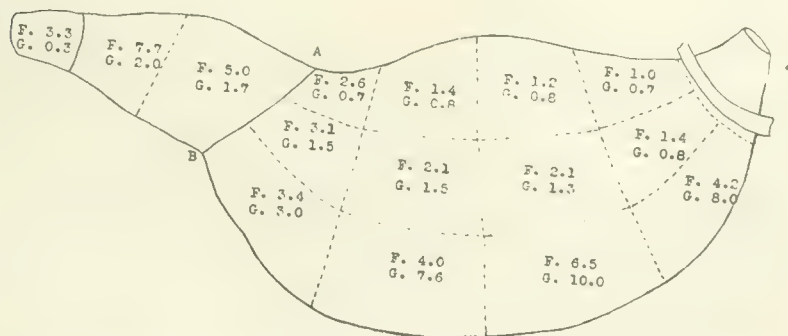


FIG. 2. Anterior surface of the stomach of the cat showing the different regions stimulated with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach. The other unbroken line represents the pylorus.

achs, no reactions could be obtained at all with the strengths of current used. This comparative sluggishness of the greater curvature was more marked with the galvanic than with the faradic current. Experience showed that if no reaction could be seen after ten seconds, it was useless to stimulate further. The graphic records, however, suggested very strongly that such apparently negative results were more often due to the very small amplitude of the contraction rather than to an entire absence of response. It seemed best, therefore, in making the averages, to represent these results by 10 seconds, assuming that some reaction had occurred by that time. This has given results quite comparable to those found in any single stomach of moderate irritability.

Turning to the charts again, it will be seen that the latent period becomes longer as we approach the antrum pylori. Although the muscle begins to thicken in the preantrum, there is, in the rabbit, a very definite transition from the body of the stomach to the antrum. This can be brought out markedly by leaving the stomach 48 hours in ice-cold Ringer's solution. The dividing line is then very sharp between the gray-blue of the body of the stomach and the red muscle in the pyloric portion. This division is not so apparent in the cat and dog, but it can easily be demonstrated. In all the animals studied, the change in the type of reaction to stimuli was equally sharp at this line. In the preantrum there were deep hour-glass contractions; in the antrum, a centimeter away, there were peculiar local puckerings and tumefactions, often followed by a spasm of the whole pyloric end of the stomach. The latent period for the faradic current was often more than double that observed on the other side of the dividing line. Strange to say, there was no such increase with the galvanic current. This rather striking observation will be discussed later. The lesser curvature reacted a little more promptly than did the rest of the antrum.

It should be emphasized that the pyloric ring showed a shorter latent period than did the rest of the antrum. The duodenum, a few millimeters away, reacted to the galvanic current very promptly—within 0.5 to 1.0 second. For some unknown reason, the duodenum seemed to lose its irritability to the faradic current very rapidly after excision. With the organs *in situ*, both currents caused the duodenum to contract promptly.

POSTERIOR SURFACE OF THE STOMACH

It was soon noticed that in most stomachs the latent periods were a little longer on the posterior than on the anterior surface, and this conclusion was supported by the averages. This sluggishness was most marked along the greater curvature. The sequence of the four series of tests—with faradic and galvanic currents on both surfaces—was changed daily, although it was soon evident that the irritability of the stomachs remained quite constant for a period longer than the hour required to complete the work. To make sure that differences in thickness or resistance in the peritoneal coat could have nothing to do in altering the reactions, tests were made here and there before and after laying bare the muscle. No differences in latent period could be produced in this way.

EFFECTS OF MECHANICAL STIMULI

Experiments in which the excised stomach was pinched by fine mouse-tooth forceps gave results very similar to those with a weak faradic current. In the rabbit, the region about the greater curvature from the fundus to a point opposite the incisura angularis failed to react. The antrum showed the typical puckering contractions after a latent period of from 3 to 5 seconds. The pylorus and duodenum reacted almost immediately. The region around the cardia was so sensitive that a contraction generally appeared each time the Ringer's solution was poured on the stomach.

DIFFERENCES IN IRRITABILITY

With weaker currents, a close relation could be shown between the degree of irritability and the latent period. With one-fourth the strength of the galvanic current previously used, reactions were obtained (in the rabbit) only around the cardia; with one-half the strength, they appeared within a radius of 3 cm. from the junction of the esophagus and lesser curvature. This also was the only part of the stomach that reacted to the faradic current with the secondary coil at 13 cm. Similar results were obtained in the cat and dog.

Just as in the frog, stimulation, particularly of the middle region of the stomach, often showed itself first as a contraction at the cardia or along the lesser curvature. This might or might not be followed by a contraction at the point stimulated, depending on the strength and duration of the stimulus. This was very marked in the cat.

STUDIES WITH THE STOMACH IN SITU

To make sure that these differences were not due to the trauma of excision or to unequal post-mortem changes in the gastric plexuses, the work was repeated on the stomachs of rabbits, cats and dogs whose abdomens were opened after etherization. The vagi and splanchnics were left intact. The intestines were covered with gauze wrung out in warm Ringer's solution, and the stomach wall was moistened frequently with the same fluid. Regional differences were found corresponding to those in the excised stomachs. The cardia, lesser curvature and pyloric antrum reacted in about the usual time; but, strange to say, the rest of the stomach—the fundus and body—was generally much less irritable than in the excised organ, and often this region would not react at all to the currents used. The possible significance of these findings will be discussed later.

EXPERIMENTS ON THE HUMAN STOMACH

A few experiments performed on the human stomach, while necessarily inconclusive, suggest that similar differences will be found there also. Through the kindness of Doctors Baxter and Brill, I had the opportunity of making the desired tests twenty minutes after death in a man who died of nephritis. Unfortunately, there was ascites with edema of the peritoneum which may have had some influence on the reactions. Both in situ and when excised, this stomach was less irritable than those of animals, and to get reactions with the faradic current, the secondary coil had to be at 6 cm. The cardia and adjacent lesser curvature could not be studied satisfactorily in situ as the pulling that was necessary seemed to affect the reactions. Elsewhere the reactions were very much like those in the cat's stomach. After excision, the stomach contracted down to the cylindrical J form, so well known to radiologists. It seemed to lose its irritability rapidly: the preantral region was the only place which reacted twice in the same way, and no conclusions could be drawn.

Another specimen obtained at operation on a man with beginning malignant degeneration of a small scar at the pylorus showed definitely the difference between faradic and galvanic effects in the antrum; also the much greater irritability of the duodenum as compared with the stomach. Through the kindness of Dr. W. I. Terry, I was able to study the reactions in the pyloric half of another human stomach during an operation for an old cholecystitis. The anesthetic was gas-oxygen

and the abdominal wound was infiltrated with novocain. The only definite statements that could be made after this work were that the stomach was less irritable than that of the animals; that it was a little more irritable near the lesser than along the greater curvature, and that the irritability of the duodenum was much greater than that of the stomach. Even with the coil at 4 cm., the faradic current failed to bring about a contraction in the stomach.

DISCUSSION

The lesser curvature, and particularly that part near the cardia, is more irritable than the fundus and body of the stomach; and it seems very probable that these differences can be ascribed to the developmental peculiarities outlined at the beginning of this paper. Other differences have been found, however, which show that the distance, spacially or embryologically, from the primitive tube is not the only factor modifying the irritability. As was suggested in the previous paper (10), the muscle of the stomach wall has probably been modified to serve different purposes in different regions. This would explain the big differences which I believe exist between the muscle of the body of the stomach and that of the pyloric antrum. Such differences seem inevitable when we remember the differences in function. While the principal activity of the fundus is to maintain a slight even pressure on its contents, the antrum must contract rhythmically and powerfully for hours at a time. Recently Lee, Guenther, and Meleney (11) have shown how remarkably the diaphragm differs from other muscles in its physiologic reactions and its chemical constitution. These differences are all related to its unceasing activity. For the same reason, we may expect some day to find that the reddish muscle in the pyloric antrum has more sarcoplasm, glycogen, etc., than the pale muscle in the fundus.

The better reaction of the pyloric antrum to the galvanic current suggests that the muscle is more sluggish in this region. One is reminded of the reaction of degeneration in striated muscle, or of such experiments as those of Fick (12) who noticed that an induced current which stimulated a frog's muscle had no effect on a mussel's muscle. The latter responded, however, to a slowly increasing galvanic current which had no effect on the frog. Further experience with excised strips has given me the impression that this sluggishness is due partly to nervous inhibition. If the irritability of the muscle is dependent upon

its nervous connections, we should expect the reactions to become progressively slower after removal of the stomach from the animal, and particularly after the trauma of cutting strips. The opposite is true; not only may the latent period become shorter, but it has been found in a number of instances that strips from the antrum would contract more promptly after they had been in the ice-box 24 and even 48 hours than they did immediately after excision. It has already been noted that the excised strips will generally beat rhythmically on the second or third day better than on the first. Although the enteric nerves are probably the last to die in the body (13), it is hardly conceivable that their functional capacity could *improve* after 48 hours, and we must look for the differences in the muscle itself.

The nervous inhibition with the stomach *in situ* probably explains Meltzer's failure to get good reactions from the cardiac half of the stomach. As will be shown in a subsequent paper, strips of muscle cut from such a refractory fundus and stimulated in a moist chamber will react markedly to the same current after a latent period of less than one second. Ducceschi has remarked upon the refractoriness of the pyloric region when it is active. It would appear that the nervous mechanism serves more to restrain the muscle than to raise its irritability. Such inhibition may be very necessary to save the stomach from the fatigue and perhaps incoordination that might follow its reaction to every stimulus received during digestion.

It is very probable that there are big differences again in the muscle of the duodenum, which not only contracts so differently, but has a shorter latent period and a greater irritability than that of the antral muscle. This was noticed by Meltzer. Schiff also remarked, years ago, that when the digestive tract is stimulated actively by the cutting off of its blood supply, the stomach, with the exception of the cardia, is the part least affected (14).

The tendency of the antrum to respond as a whole when stimulated at any one spot may have some clinical interest. Roentgenologists occasionally see such spasm in man when an ulcer is located in the pyloric region. As the pylorus is more irritable than the rest of the antrum, it is not surprising that it should often be closed so tightly when this part of the stomach is irritated. Interesting also to the clinician is the tendency to deep hour-glass contraction after stimulation in the preantral region in animals. That is the common location for such spasm in man. This finding is in agreement also with the observation that strips from this region show a far wider amplitude of

rhythmic contraction than do those from any other part of the stomach wall.

I agree entirely with Meltzer that it is quite hopeless to attempt to influence the stomach therapeutically by intragastric or abdominal electricity. The refractoriness of the stomach *in situ*, particularly when stimulated through the mucous membrane, is so great that even powerful currents will produce no motor effect.

SUMMARY

It has been shown in the frog's stomach that the cardia is the most irritable region; also that the latent period is shortest at the cardia and longest near the pylorus. These peculiarities should have much to do with insuring the aboral course of the peristaltic waves.

The mammalian stomach has been studied from the point of view that it has developed from a primitive tube much as the heart has been enlarged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature. These developmental differences probably have something to do with the regional differences in irritability and latent period found in the stomachs of rabbits, cats and dogs.

The latent period for faradic, galvanic and mechanical stimuli is shortest around the cardia and along the lesser curvature as far as the incisura angularis. These limits include also the most irritable part of the stomach. The region of the greater curvature and fundus is much less irritable and often fails to react at all.

There is, particularly in the rabbit, a definite transition from the pale muscle of the body of the stomach to the reddish muscle of the antrum. The change in the type of reaction to stimuli is equally sharp at this line. In the preantrum, there are deep hour glass contractions; in the antrum, a centimeter away, there are small puckerings often followed by spasm of the whole pyloric end. The latent period in the antrum is markedly lengthened for the faradic current: very slightly for the galvanic.

The pyloric ring is more irritable and reacts more promptly than does the rest of the antrum.

The duodenum is much more irritable than the pyloric antrum.

The posterior surface of the stomach is a little less irritable than the anterior surface, and the latent periods are longer.

With the exception of the cardia and lesser curvature, the stomach

in situ is often quite refractory to stimulation in its cardiac half. This seems to be due to nervous inhibition as it is less marked in the excised stomach, and still less so in strips of muscle cut from the fundus or greater curvature.

It seems likely that the nervous mechanism serves more to restrain the muscle—to keep it from reacting to every stimulus—than to render it more irritable.

The little work done so far on the stomach of man has given results in agreement with those obtained in animals.

BIBLIOGRAPHY

- (1) ALVAREZ: *This Journal*, 1916, xl, 585.
- (2) MELTZER: *N. Y. Med. Journ.*, 1895, lxi, 746.
- (3) LÜDERITZ: *Arch. f. d. ges. Physiol.*, 1891, xlix, 158.
- (4) DUCCESCHI: *Arch. p. l. Sci. Med.*, 1897, xxi, 121.
- (5) ROGERS AND HARDT: *This Journal*, 1915, xxxviii, 284.
- (6) CARLSON: *This Journal*, 1913, xxxii, 263.
- (7) MAY: *Journ. Physiol.*, 1904, xxxi, 264.
- (8) BARBERA: *Zeitschr. f. Biol.*, 1898, xxxvi, 239.
- (9) PORTER: *This Journal*, 1902, viii, xxxv.
- (10) ALVAREZ: *This Journal*, 1916, xl, 597.
- (11) LEE, GUENTHER AND MELENEY: *This Journal*, 1916, xl, 446.
- (12) FICK: Quoted from Winkler *Arch. f. d. ges. Physiol.*, 1898, lxxi, 358.
- (13) CANNON AND BURKET: *This Journal*, 1913, xxxii, 347.
- (14) SCHIFF: *Lecons sur la Physiol. d. l. Digestion*, Paris, 1867, ii, 314.

ICTERUS.

A RAPID CHANGE OF HEMOGLOBIN TO BILE PIGMENT IN THE PLEURAL AND PERITONEAL CAVITIES.

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In an earlier communication (1) we have been able to show that bile pigment could be formed from hemoglobin without the agency of the liver. Solutions of hemoglobin were introduced into the blood vessels of dogs whose livers had been excluded from any part in this reaction. There was a prompt formation of bile pigment from hemoglobin with no possible direct liver action. This transformation can take place within a space of two hours when active circulation is maintained in the head and thorax alone. It seemed probable that the endothelium might be the tissue whose activity was responsible for this change of hemoglobin to bile pigments.

This work has received confirmation from experiments of McNee (2) who repeated the experiments of Minkowski and Naunyn with geese. He found that icterus did develop without liver activity and found evidences of endothelial activity. He suggests (3) that the Kupffer cells may normally transform hemoglobin to bile pigments.

The experiments given below prove conclusively that still other tissues can rapidly transform the hemoglobin pigment into bile pigment. There can be no question of any direct liver activity in these experiments. Some transformation may take place within eight hours, but the pigment production in twenty-four hours can usually be estimated with considerable accuracy. This mesothelium of the serous cavities can not effect this transformation as promptly as it is effected in the circulating blood, but the contact with the living pleural cells is not as active and intimate,—not a circulatory contact.

If we assume that the capillary endothelium can transform hemoglobin to bile pigment, it is at once obvious how intimate is the contact of the circulating hemoglobin solution with the living protoplasm of the vessels. The solution of hemoglobin in the serous cavities may be in more or less motion (respiration, peristalsis, or body movements), but at best it is not in very intimate contact with the living protoplasm. The wonder is that the transformation is so prompt and easily recognizable. It is impossible to estimate the relative activity of endothelium and mesothelium, but there can be little difference if we allow for the rapidity of circulation and surface contact.

It has been claimed by older workers (Virchow (4) and many others) that blood standing long in contact with living tissues was slowly changed to a golden pigment hematoidin which is chemically equivalent to bilirubin. Virchow used the term hemolytic icterus for this reason. Guillain and Troisier (5) noted in human cases with pleural hematomas that bile pigment was formed after a considerable period with no complicating liver abnormality. Von der Bergh and Snapper (6) were able to find greater amounts of bile pigment in serous exudates than in the blood serum, and they speak in favor of the extrahepatic formation of bile pigment. That the serous cavities can rapidly transform hemoglobin to bile pigment has not been hitherto recognized.

EXPERIMENTAL OBSERVATIONS.

The experiments are not all given in detail, but the essential facts are given in Tables I and II. A typical experiment from each group is given in sufficient detail, and it is to be emphasized that the majority of these experiments caused no inflammation or even permanent injury to the pleural or peritoneal cavity. In a few cases due to slips in technique there developed a pleurisy or a peritonitis, but these complications did not modify the transformation of hemoglobin to bile pigment. The crystalline hemoglobin causes a slight irritation in the pleural or peritoneal cavity, and it is possible that the inflammatory reaction may assist in the transformation, but it is not an essential factor.

Method.

Active and vigorous dogs were used in all of our experiments. The urine was obtained by catheter, and in every case it was examined before the experiment. The Huppert-Salkowski test for bile pigments was used in examining the urine, and unless otherwise stated 20 cc. of urine were employed in the test. Either fresh or crystalline dog hemoglobin was used. The fresh hemoglobin was obtained by laking washed erythrocytes with distilled water.

The preparation of crystalline hemoglobin was carried out according to the method of Bradley and Sansum (7). The red blood cells obtained from defibrinated dog blood by centrifugalization were washed eighteen or twenty times with normal salt solution, then they were laked with toluene and a little distilled water. The toluene layer was removed and the solution centrifugalized and decanted from the stroma material. This hemoglobin solution was then mixed with 20 per cent by volume of toluene, shaken thoroughly, and set aside in the cold. After twenty-four or forty-eight hours the mixture is nearly a solid mass of large well formed crystals. The crystals are collected by centrifugalization, washed with cold water, spread thin on glass, and dried in a stream of warmed air. Fresh 0.6 per cent salt solution was used as a diluting medium for the fresh or crystalline dog hemoglobin.

The fluids were introduced into or withdrawn from the pleural or peritoneal cavity through a trocar. After withdrawing the pleural or peritoneal fluids they were centrifugalized immediately, the supernatant fluid was decanted and made definitely alkaline by the addition of a saturated solution of sodium carbonate, then a 10 per cent solution of calcium chloride was added until precipitation was complete. After centrifugalization at high speed the supernatant fluid was again poured off, and the calcium bile pigment compound remained as a yellowish precipitate. The precipitate was washed free from other coloring matter and collected again by centrifugalization. Finally the precipitate dissolved in a hot solution of 5 per cent hydrochloric acid in 95 per cent alcohol gave the characteristic blue green color when bile pigments were present.

The method used to determine the amounts of bile pigments quantitatively will be published in the near future. This method in abstract consists of reading the blue green color of the acid alcohol extract in a fixed dilution against a permanent wedge of similar color which has been standardized against chemically pure bilirubin solutions of known amounts. The common sulphonephthalein colorimeter may be used to advantage.

Pleural Experiments.

Bile Pigments Formed from Hemoglobin in Periods of 8 to 66 Hours.

Dog 15-11.—8 and 24 hours. (Table I.) Short haired mongrel, male; weight 14 pounds.

Mar. 17. Dog is normal. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10 a.m. 20 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 500 cc. of sterile 0.6 per cent salt solution. 11.10 a.m. No respiratory embarrassment. 2 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6 p.m. 100 cc. of dark red fluid removed from right pleural cavity are negative for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin.

Mar. 18, 9.35 a.m. Dog is very active. Rectal temperature 39.1°C . 20 cc. of urine negative for bile pigments and hemoglobin. 10 a.m. 158 cc. of dark red fluid recovered from right pleural cavity are positive for bile pigments. 11.20 a.m. Right pleural cavity irrigated with 500 cc. of 0.6 per cent salt solution. Appetite is fairly good.

Mar. 19, 9 a.m. Dog is listless. Rectal temperature 40.2°C . Respiratory movements over right thorax are impaired.

Mar. 20, 10 a.m. Rectal temperature 39.1°C . Dog is very active and eats well. Respiration normal.

Dog 15-34.—8 hours. (Table I.) Mongrel setter, male; weight 55 pounds.

May 12. Dog is active and vigorous. 8.30 a.m. 20 cc. of urine are negative for bile pigments. 8.45 a.m. 500 cc. of water are introduced into the stomach through a stomach tube. 9.30 a.m. 30 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 1,500 cc. of sterile 0.6 per cent salt solution. 11 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. 1,175 cc. of dark red, turbid fluid recovered from right pleural cavity. Chemical test is positive (suspicious) for bile pigments. 5.50 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Drinks some milk.

May 13, 9.30 a.m. Dog is listless and refuses to eat. Rectal temperature 102.3°F . Respiratory movements over right thorax are impaired, and a friction rub is palpable.

May 14, 10 p.m. Very active. Rectal temperature 101.8°F . Respiratory movements are normal.

Dog 15-39.—17 and 66 hours. (Table I.) Black mongrel, female; weight 32 pounds.

May 10. Dog is in excellent condition. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 11.15 a.m. 30 cc. of washed, laked red blood cells suspended in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.20 p.m. 930 cc. of dark red fluid, recovered from the right pleural cavity, give a positive (suspicious) test for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Respiratory movements over right thorax are somewhat delayed.

May 11. Dog is active and vigorous. Rectal temperature 102.6°F . Respiratory movements are normal.

May 17. Dog is in excellent condition. Weight 32 pounds. 3.30 p.m. 20 cc. of urine are negative for bile pigments. 5 p.m. 30 cc. of washed, laked red blood cells are introduced into the left pleural cavity with 1,000 cc. of 0.6 per cent salt solution. 6.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. Respiratory movements are somewhat delayed over left thorax.

May 18. 9.30 a.m. Rectal temperature 103.2° F. Respiratory movements are markedly delayed over left thorax. 20 cc. of urine are negative for bile pigments and hemoglobin. 10 a.m. 390 cc. of dark red fluid recovered from the left pleural cavity contain 0.14 mg. of bile pigment. 5.30 p.m. Animal is quite active.

May 19. Dog is not very lively. 9 a.m. Rectal temperature 102.8° F.

May 25. Weight 32 pounds. Dog is active and vigorous. Rectal temperature 102.2° F. 4.30 p.m. 20 cc. of urine are negative for bile pigments. 4.45 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 4.50 p.m. The right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution. The fluid recovered is water-clear; bile pigment test negative. 6.10 p.m. 10 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of 0.6 per cent salt solution are introduced into the right pleural cavity. 6 p.m. Respiratory distress is not marked.

May 26. Dog is quite active and eats well. The movements of the right thorax are somewhat impaired. Rectal temperature 101.8° F. 2.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.

May 27. Rectal temperature 102.2° F. Movements of the right thorax are markedly impaired. Refuses food. 12.35 p.m. 20 cc. of urine are faintly positive for bile pigments.

May 28. Rectal temperature 102.8° F. 10.30 p.m. 20 cc. of urine are faintly positive for bile pigments. 11 a.m. 510 cc. of dark red turbid fluid recovered from the right pleural cavity contain 5.40 mg. of bile pigments. 11.20 a.m. Right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution.

May 29. Dog is normal.

Dog 15-52.—24 hours. (Table I.) Bull terrier mongrel, male; weight 41.5 pounds.

June 16. Dog is normal. 10 p.m. 20 cc. of urine are negative for bile pigments. 11.35 a.m. 9 gm. of crystalline dog hemoglobin dissolved in 1,000 cc. of 0.6 per cent salt solution are injected into the right pleural cavity. 12 m. Respiratory distress is not marked. 6 p.m. Animal drinks some milk.

June 17. Dog is quite active. Respiratory movements over right thorax are impaired. Rectal temperature 102.8° F. 10 a.m. 20 cc. of urine are negative for bile pigments. 11.30 a.m. 575 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.18 mg. of bile pigment. 5 p.m. Respiratory movements are quite normal.

June 18. Dog is very active. Rectal temperature 102.4° F.

TABLE I.

Hemoglobin Changed to Bile Pigment in Pleural Cavity.

Dog No. Date.	Weight	Pleural cavity.	Time in pleural cavity	Bile pigment tests.	Bile pigments	Fluid intro- duced.	Fluid recov- ered	Bile in urine.		Remarks.
								Before.	After.	
1915	lbs.		hrs.		mg.	cc	cc.			
15-11										
Mar. 17	14.0	Right.	8	0	0	620	100	0	0	Fresh dog hb.
15-11*										
Mar. 17	14.0	"	24	+	—	620	158	0	0	" " "
15-34										
May 12	55.0	"	8	(?)+	—	1,580	1,175	0	0	" " "
15-39										
May 10	32.0	"	7	(?)+	—	1,160	930	0	0	" " "
15-40										
May 10	22.0	"	6	(?)	—	700	555	0	0	" " "
15-41										
May 12	36.0	"	8	(?)+	—	1,480	1,230	0	0	" " "
15-39										
May 17	32.0	Left.	17	+++	0.14	1,105	390	0	0	" " "
15-40										
May 17	21.5	"	18	+++	0.09	805	265	0	0	" " "
15-52										
June 16	41.5	Right.	24	++++	0.18	1,000	575	0	0	Crystalline dog hb., 10 gm.
15-54										
June 16	36.5	"	24	++	—	800	395	0	0	" " " 7 "
15-53										
June 16	29.5	"	25	+	—	800	408	0	0	" " " 7 "
15-56										
June 16	39.0	"	25	++	—	900	318	0	0	" " " 7 "
15-41										
May 20	36.5	Left.	43	+++	0.15	805	254	0	+	Fresh " "
								(faint)		
15-43										
May 20	34.0	Right.	44	++++	0.36	1,100	808	0	0	Crystalline " " 4 "
15-40										
May 25	22.0	"	65	++++	3.60	940	430	0	+	Fresh " "
								(faint)		
15-39*										
May 25	32.0	"	66	++++	5.40	1,100	510	0	+	Crystalline " " 10 "
								(faint)		

* See history for details.

Dog 15-43.—43 hours. (Table I.) Mongrel spaniel, male; weight 34 pounds.

May 20. Dog is in excellent condition. Rectal temperature 102° F. 12.10 p.m. 20 cc. of urine are negative for bile pigments. 12.15 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 3.10 p.m. 4 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.

May 21. Dog is active and eats well. Respiratory movements over the right thorax are impaired. Rectal temperature 102.2° F.

May 22. Rectal temperature 103.1° F. 10.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 11 a.m. 808 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.36 mg. of bile pigments.

May 23. Rectal temperature 101.8° F. Dog is normal.

The preceding experiments are conclusive proof that mesothelium can rapidly transform hemoglobin into bile pigment. The lining cells of the pleural cavity like other body cells (probably endothelium) can form bile pigments out of hemoglobin, either freshly laked or crystallized. This solution of hemoglobin is not in very intimate contact, not in circulatory contact, as is hemoglobin in contact with the endothelium of the capillaries, yet the transformation takes place promptly in a few hours. There is experimental evidence of this bile pigment transformation in the pleura within eight hours, but a very definite amount is formed within eighteen to twenty-four hours, an amount often sufficient for accurate determination.

Dog 15-20.—8 hours. (Table II.) Mongrel poodle, male; weight 19.5 pounds.

Mar. 29. Dog is normal. 10 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10.30 a.m. 35 cc. of washed, laked red blood cells are introduced into the peritoneal cavity with 800 cc. of 0.6 per cent salt solution. 12.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2 p.m. Rectal temperature 101.3° F. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6.30 p.m. 300 cc. of dark red turbid fluid recovered from the peritoneal cavity give a questionably positive test for bile pigments. 6.45 p.m. Peritoneal cavity is irrigated with 2,000 cc. of 0.6 per cent salt solution. Urine gives positive (suspicious) test for bile pigments.

Mar. 30. Dog is very lively and eats well. Rectal temperature 101.7° F.

Dog 15-43.—28 hours. (Table II.) Mongrel spaniel, male; weight 33.5 pounds.

June 17. Dog is in excellent condition. 9.45 a.m. 20 cc. of urine are negative for bile pigments. 11.15 a.m. 7 gm. of crystalline dog hemoglobin dissolved in 2,800 cc. of 0.6 per cent salt solution, introduced into the peritoneal cavity.

12 m. Dog shows no embarrassment. 5.30 p.m. Animal listless and refuses to eat. Considerable rigidity of abdomen.

June 18. Rectal temperature 103.8° F. Animal is listless, will not eat, and lies quietly in cage. Abdominal muscles are very tense. 2 p.m. 20 cc. of urine are positive for bile pigments. 3.20 p.m. 755 cc. of dark red turbid fluid recovered from the peritoneal cavity give a positive test for bile pigments. 3.35 p.m. Peritoneal cavity irrigated with 3,000 cc. of sterile 0.6 per cent salt solution. 5 p.m. Rectal temperature 104.2° F.

June 19. 9 a.m. Animal is found dead. Autopsy showed a diffuse fibrinous peritonitis. Other organs are negative.

Dog 15-55.—48 hours. (Table II.) Mongrel hound, male; weight 40.5 pounds.

June 16. Dog is in excellent condition. 2 p.m. 20 cc. of urine are negative for bile pigments. 2.40 p.m. 7 gm. of crystalline dog hemoglobin dissolved in 3,000 cc. of 0.6 per cent salt solution introduced into the peritoneal cavity. 6 p.m. Dog is quite active. 20 cc. of urine are negative for bile pigments and hemoglobin.

June 17. Rectal temperature 102.2° F. 10.10 a.m. 20 cc. of urine are positive for bile pigments. Hemoglobin negative.

June 18. Dog is very lively and eats well. 9.30 a.m. Rectal temperature 103.6° F. 1.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2.30 p.m. 310 cc. of dark red, turbid fluid recovered from peritoneal cavity contain 0.27 mg. of bile pigment. 2.50 p.m. Peritoneal cavity irrigated with 2,000 cc. of 0.6 per cent salt solution. 5.30 p.m. Dog is very active.

June 30. Dog is normal.

TABLE II.

Hemoglobin Changed to Bile Pigment in Peritoneal Cavity.

Dog No. Date.	Weight	Time in periton- eal cavity.	Bile pigment tests.	Bile pigments	Fluid intro- duced.	Fluid recov- ered.	Bile in urine.		Remarks.
							Before	After.	
1015	lbs.	hrs.		mg.	cc.	cc.			
15-20									
Mar. 29	19.5	8	(?)		985	300	0	(?) +	Fresh dog hb.
15-43									
June 17	33.5	28	+	—	2,800	755	0	+	Crystalline dog hb., 7 gm.
15-34									
May 20	56.0	44	++++	0.25	4,000	1,360	0	(?) +	" " " 7 "
15-41									
June 17	36.5	48	++++	0.18	3,000	310	0	+	" " " 7 "
15-57									
June 16	24.5	48	++++	0.32	2,500	255	0	+	" " " 7 "
15-55									
June 16	40.5	46	++++	0.27	3,000	310	0	+	" " " 7 "

The preceding experiments and Table II show that the peritoneal cavity can transform hemoglobin into bile pigment with the same promptness as the pleural cavity. The two cavities are lined by a similar mesothelium, so there need be no surprise when the reactions are found to be similar. The difference in absorption from the two cavities is obvious. The pleural cavity gives slow absorption, most of the fluid introduced is recovered, and little if any of the formed bile pigment appears in the urine. The peritoneal cavity gives a pretty rapid absorption, most of the fluid introduced is taken up, and the bile pigments appear in the urine as a result.

DISCUSSION.

A difference in absorption from the pleural and peritoneal cavities has been noted and it comes out clearly in Tables I and II. The relatively rapid absorption from the peritoneal cavity causes the escape of bile pigments in the urine. Even in the pleural experiments, however, there is considerable absorption which indicates that in both pleural and peritoneal cavities there is an outflow of fluid. Whether there is interchange between the introduced fluid and the body fluids is uncertain, but much interchange is unlikely. In these experiments the dogs were all normal and their urine free from bile pigment at the beginning of the experiments, which means that the serum was negative to bile pigments by the test employed. That the blood pigments could have been absorbed into the blood, changed to bile pigments in association with the liver, and again diffused into the pleural fluid, is inconceivable. The observations in the pleural experiments (Table I) show that the urine was free from hemoglobin and bile pigments during the first forty-eight hours.

A theoretical objection could be raised to the experiments with the hemoglobin solutions in the peritoneal cavity. It could be argued that this solution comes in contact with the liver and could be modified in this manner. The solution does come in contact with the serous covering of the liver, but not with the hepatic epithelium. Diffusion through the serous epithelium of the liver is conceivable, but most improbable, and the pleural experiments are not open to such an objection.

The use of crystalline hemoglobin seems to cause more irritation of the serous surfaces than does a fresh solution of hemoglobin obtained from freshly laked red corpuscles. When this irritant action lasts over a period of three days there is undoubtedly some new formation of capillaries and proliferation of mesothelium, as well as an escape of various wandering cells. This reaction brings other extrahepatic factors into the equation and apparently accelerates the formation of bile pigment (Table I).

All this evidence indicates that the function of changing hemoglobin to bile pigment is not limited to any single cell or even to two types of cell. It may well be a function of endothelium and mesothelium as well as of hepatic epithelium. Perhaps wandering cells have this property of transforming blood to bile pigment. Many of these wandering cells probably have their origin from endothelium, and may they not retain this functional capacity? It is quite possible that other epithelium besides liver epithelium may be able to effect this transformation. More experiments are being carried out with this point in view. If we go a step further it may be suggested that living protoplasm in general can change hemoglobin to bile pigments.

Still other questions may be raised. Can tissue juices or ferments bring about this transformation of hemoglobin to bile pigment? We have performed many experiments with negative results, but have not yet given up the attempt although success seems unlikely.

CONCLUSIONS.

It is known that hemoglobin can be rapidly changed to bile pigment in a circulation confined to the head, neck, and thorax. This excludes direct liver participation (1).

These experiments show that hemoglobin can be changed to bile pigment within the pleural or peritoneal cavities.

This transformation can usually be detected after eight hours, and the amount can often be estimated quantitatively after an interval of twenty-four hours.

Such experiments demonstrate the importance which may attach to extrahepatic bile pigment formation. That bile pigments can be formed without direct liver activity is established beyond doubt.

It is highly probable that endothelium as well as mesothelium (serous cavities) can transform hemoglobin into bile pigment. It is possible that this property may reside in cell protoplasm in general.

BIBLIOGRAPHY.

1. Whipple, G. H., and Hooper, C. W., *Jour. Exper. Med.*, 1913, xvii, 612.
2. McNee, J. W., *Med. Klin.*, 1913, ix, 1125.
3. McNee, J. W., *Jour. Path. and Bacteriol.*, 1913-14, xviii, 325.
4. Virchow, R., *Virchows Arch. f. path. Anat.*, 1847, i, 379.
5. Guillaïn, G., and Troisier, J., *Rev. de. med.*, 1909, xxix, 465.
6. von der Bergh, A. A. H., and Snapper, J., *Berl. klin. Wchnschr.*, 1914, li, 1109.
7. Bradley, H. C., and Sansum, W. D., *Jour. Biol. Chem.*, 1914, xviii, 497.

BILE PIGMENT METABOLISM

I. BILE PIGMENT OUTPUT AND DIET STUDIES

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In the following communications we propose to show that the bile pigment secretion can be influenced at will by modification in the diet. This means probably that the liver has a *constructive* function in forming bile pigments as well as the accepted eliminative function which depends on the destruction of red cells containing hemoglobin. The statement that bile pigment elimination may be influenced by dietary conditions is proved conclusively for dogs, and there is every reason to suppose that it is true for other animals.

We realize that the above statements are contrary to the accepted views of physiologists, and will require very convincing proof which we submit in detail below. The generally accepted theory covering the life history of the bile pigments may be sketched somewhat as follows. Degeneration of red cells frees hemoglobin which is brought to the liver and there changed to bile pigments which are excreted as waste products into the intestine. Here the bile pigments are reduced to urobilin or stercobilin some of which may be absorbed and returned to the liver and again thrown out in the bile or destroyed. Some of this urobilin may escape the liver and appear in the urine, especially when the liver is not functioning normally.

We hope to add several factors to this relatively simple equation which may throw more light on the functional capacity of the liver as well as the cycle of pigment metabolism in the body. We have established (12) the fact that hemoglobin can be rapidly changed to bile pigment in the body circulation outside of the liver. McNee (5) has recently confirmed this observation. We (4) have also shown that the pleural and peritoneal cavities can rapidly transform hemoglobin into bile pigment. We believe that this extrahepatic transformation of hemoglobin into bile pigment may be more important than is generally

supposed, particularly in diseased conditions associated with icterus or hemoglobinemia.

We (11) have recorded the observation that an Eck fistula dog with obstructed common bile duct will develop icterus to a much less degree than a normal dog with obstructed duct. It has been proved that an Eck fistula liver is smaller than a normal liver and has less functional capacity (13 and 14). The suggestion is obvious that the production of bile pigments is in part due to the functional activity of the liver and not solely to the hemoglobin destruction. In this manner were we lead into a study of bile secretion and its many difficult problems.

An immense amount of work has been done upon the secretion of bile, and it is fair to say that most of it was not done in a critical spirit but apparently with the idea of proving that some one or more substances were cholagogues. Stadelmann (7 and 8) and his co-workers are among the few who emphasize the normal variations in bile flow in dogs and the extreme care necessary in drawing any conclusions from small fluctuations in bile excretion. Their experimental observations are carefully made, suitable controls are furnished, and great care is used in the analysis of figures. Their work is to be recommended as an example for all workers in this difficult field.

"Bile circulation" has been definitely established—Stadelmann (9). This means that the *bile salts* are poured into the intestine, partially absorbed from it and again excreted in the bile. This fact comes in with the observation that bile or bile salts are active cholagogues, in fact the only substances upon which there is agreement among experimental workers. Among the dozens of other drugs used as cholagogues one can pick out any single drug and find in the literature several workers who claim to show that it is a cholagogue and again the same number who claim as proven from their work that it has no cholagogue action. Almost all of the conservative workers agree that whole bile or bile salts alone give definite acceleration to the flow of bile.

"Bile circulation" for the *bile pigments* has been claimed but never demonstrated. Stadelmann (9) says that a very small amount of bile pigment *may* be absorbed from the intestine but there is no definite proof for this, and he leaves the question open. There is no question that the liver can pick bile out of the circulating blood and rapidly excrete it through the gall ducts. This applies to foreign bile as Wertheimer (10) showed by means of sheep's bile injected into dogs. This, however, does not prove that bile pigments can be absorbed from the *intestine* and excreted again in the bile as is assumed by some writers.

Hemoglobin injected into the blood stream, peritoneum or subcutaneous tissues will cause a rise in bile pigment output from the liver. Stadelmann and his pupils submit the best experiments on this point, but they do not claim that it is quantitatively eliminated as do Prugsch and Yoshimoto. The published data of Brugsch and Yoshimoto (2), Brugsch and Kawashima (3) do not establish their claims that hematin is quantitatively eliminated as bile pigment. But grant for the sake of argument that hematin is quantitatively eliminated as bile pigment, one is surprised at their argument therefrom that hemoglobin is the source of the bile pigment, and from the bile pigment one can compute the life cycle of the red cells! There may be a dozen substances which may be quantitatively or partially changed into bile pigments or the liver cells may be capable of building up bile pigments from various "building stones."

Some work has been done upon the bile pigment excretion as influenced by the injection of hemoglobin, bile and bile pigments, also various poisons known to injure the liver or destroy red blood cells (Stadelmann, Brugsch, Wertheimer, etc.). So far as we know no worker has followed the curve of bile pigment excretion with suitable control of general condition and weight of dog, hemoglobin estimation and red cell counts, the presence or absence of pigments in the urine and above all, the diet. For the present we shall confine our attention to the bile pigment output with occasional notes concerning the volume of bile flow.

At the beginning of this work about three years ago it seemed quite necessary that the bile fistula dogs should be maintained as near to a normal healthy condition as possible. Considerable time was spent upon this point, and every sort of ration was tried out, mixtures given with fresh bile and dried bile, raw liver and cooked liver, raw and cooked meat, milk, raw eggs, butter, fats, etc. We do not wish to dwell upon negative results, but will give only a review of our positive results.

We are convinced from our work that bile is a necessary life factor for a dog fed upon any common mixed diet. There are statements in the literature Albu (1), Ransom (6) that bile secretion is not essential to health in man, but we are sceptical of such reports as careful autopsy records are not submitted. We are convinced from our series of over twenty animals that it must be very unusual for a dog to be able to survive on any ordinary diet if the bile is *completely* excluded from the intestine. In our experiments, the bile which the animal may lick from its fistula during the night is not able to maintain normal equilibrium or anything approximating it.

We wish to point out again that the common bile duct can reestablish its lumen after double ligation with silk and resection of about 1 cm. of the duct. We have reported such cases in another paper (11), and note also that this can happen in a dog with a bile fistula. A tiny fistulous tract may establish itself between the cut ends of the common duct and allow the escape under pressure of a small amount of bile into the duodenum. One such dog is included in our series of simple bile fistulas. This point must be kept in mind and the stools watched very carefully for stercobilin and at autopsy a very careful search made at the site of the section of the common bile duct. When a dog with a permanent bile fistula on an ordinary diet holds his normal weight and condition, we believe that this possibility should be considered and excluded or not by examination of the feces. Only a very small amount of bile seeping into the duodenum is required to completely change the clinical picture of wasting and general malaise to one of health and activity. Study of these repaired common ducts at autopsy shows how small an amount of bile introduced at this point is necessary for health. Introduction of bile by stomach tube, however, has no such favorable effect.

Fresh bile (pig) was tried in two bile fistula dogs with poor success. It was given once or twice daily by stomach tube. This did not prevent the usual loss of weight on a mixed diet. Recently we have had better results using fresh dog's bile mixed with the food, but some animals will not eat this mixture, and it does not have permanent effects when given once or twice daily in 25 to 50 cc. amounts by stomach tube.

Dried ox bile (two grams per day) was given in capsules to many of the dogs in our earlier experiments. This dried bile surely helps maintain a normal condition, but as a rule is not sufficient with a simple mixed diet, and most of these bile fistula dogs died after several weeks with the familiar picture of emaciation, intestinal disturbances, including much loss of blood, and stupor. Dried ox bile cannot be counted upon to replace in any satisfactory way the normal flow of bile, but it does some good in some instances.

Fresh pig's liver was tried as a diet following quite a series of unsuccessful bile fistulas which were fed bile in various ways. Marked improvement was noted with fresh liver forming a part of the diet, but the dogs soon refused to eat the raw liver. Cooked liver was then tried with the same success, and we feel very certain that bile fistula dogs can be kept in practically normal condition and weight equilibrium

on a diet of cooked liver (pig or sheep). This statement must be slightly qualified as some dogs do not react as well as the majority: again the dogs' condition may remain perfect for months with a terminal loss of ground and intoxication. The diet used in the majority of experiments was the usual mixed diet of cooked meat, bones, and bread plus 100 to 200 grams of cooked liver as indicated in the charts.

That liver feeding in dogs with complete bile fistulae is of peculiar benefit, and may maintain them in a normal condition for weeks is pretty definitely established. This liver feeding is more efficacious in most cases than feeding fresh or dried bile, and is to be considered in the treatment of certain clinical cases. What particular chemical substance in the liver is responsible for this influence on the abnormal metabolism of bile fistula dogs? We hope to give an answer to this question in the near future.

METHODS

1. Operation and post-operative care

The operative procedure and care of the animals are very important factors in these experiments, and have not been sufficiently emphasized by many workers. The object should be to maintain the dog in as near to perfect condition as possible, and this is not easy.

All operations are done under ether-morphia anaesthesia, and strong, active, short-haired dogs of about thirty pounds weight are most suitable. An incision is made in the mid line, and the gall bladder dissected free from the liver. The common duct is freed, doubly ligated, and about 1 cm. between the ligatures is resected. The gall bladder is then pulled through a small stab wound in the right rectus close to the costal margin, and fixed by silk sutures to the sheath of the rectus. The stab wound should be rather small, as a small fistula is desired. The gall bladder is then opened, and a small piece of rubber tubing about 1 cm. in diameter is pushed down into its lumen, and fixed here by two stay sutures. The median incision is closed as usual. No dressings are applied, as they only serve to irritate the skin, and the wounds heal very promptly. The tube in the gall bladder should be removed on the sixth or eighth day, and care should be taken that the tube drains freely, else a distinct icterus may result, and prolong convalescence or actually render the animal useless for further work. If there are no complications, the dog should be in good condition by the third week, and ready for bile collections by the method described below.

A regular routine is very important in the experiments, and is a part of the care so necessary to keep the dogs in good condition. The dogs are allowed to exercise in a yard for about one-half hour in the morning. They are brought in for collection of bile about 10 a.m., and put up in the harness for a period of six or eight hours, during which time specimens are removed every two hours. The dogs are fed two hours after the start of the experiment each day. At the end of the observation they are turned into the yard to exercise about one hour, and then given a heavy feed and locked in their cages. Dogs and cages are kept very clean, and dogs are washed once or twice a week. Collections are made *every* week day, and this is important, because otherwise the fistula will narrow and obstruct the outflow, jaundice will supervene, and the general condition of the dog will suffer. Great variations in the bile pigment output will then occur. It is best to dilate the bile fistula occasionally but very gently. On Sundays the fistulas are drained by a catheter, but no collections are made. Under this régime, the bile collected will be perfectly clear, except for an occasional small shread of mucus, and at autopsy the larger bile passages will be smooth, pale and normal throughout except for slight dilatation.

2. Collection of bile

A small flexible rubber tube about 7 cm. long is passed into the bile fistula, and should fit accurately. This tube passes through the short stem of a glass funnel, and is fixed firmly in it. This serves two purposes—first, the funnel catches a little mucus which oozes from the edge of the fistula, and prevents its mixture with the bile secretion; second, it will show any escape of bile about the tube which never occurs in suitable fistulae, and serves to hold the tube firmly in place in the fistula. The glass funnel is held tight against the abdomen and fistula by a binder reinforced by metal about the funnel. The binder is held accurately in position by adhesive plaster over the dog's back, and a small rubber bag is fixed to the end of the glass funnel stem to catch all the bile. Wide cloth and webbing strips are passed under the thorax and forelegs to prevent the dog from lying down on the rubber bag and spoiling the collection. The dogs stand or sit on their haunches, and doze quietly a good part of the period of collection.

3. *Bile pigment estimation*

One cubic centimeter of the bile to be analyzed is added to 49 cc. of the following solution (ethyl alcohol 95 per cent, 100 cc., nitric acid concentrated, 0.4 cc., and hydrochloric acid concentrated, 2 cc.) and mixed in a volumetric flask. The flask is shaken thoroughly, corked, and allowed to stand at room temperature about eighteen hours, when the readings are made. This solution turns bluish green, and reaches its maximum color in twelve to eighteen hours, and holds its intensity for twenty-four to forty-eight hours or longer. The solution is filtered through paper, and read in a colorimeter (Autenrieth-Königsberger as modified by Rowntree and Geraghty). The method is very simple and accurate to 0.01 mgm. of bilirubin.

The bile pigments in the serum or urine are estimated as follows. The fluid is made alkaline with a saturated solution of sodium carbonate and mixed with a 10 per cent solution of calcium chloride giving a voluminous precipitate containing the bile pigments. The precipitate is thrown down and washed repeatedly with distilled water by use of the centrifuge. The precipitate is finally dissolved in a measured amount of the nitro-hydrochloric acid alcohol solution, and allowed to stand at room temperature over night. The pigments are then estimated by the colorimeter.

For this work it is very desirable to have a permanent standard wedge for the colorimeter, and after many trials the best result was obtained as follows. A normal solution of very pure copper sulfate is treated with a few drops of a dilute watery solution of India ink. This suspension is not permanent unless fixed in some way. This is accomplished by a solution of agar-agar and gelatin which must be cleared with great care. Equal parts of the gelatin-agar solution and normal copper sulfate solution are combined while still warm, and poured into the standard wedge. The mixture of copper sulfate, ink, and gelatin-agar when it cools is permanent, and the wedge may be sealed with vaseline. Our standard wedge had been standardized against (a) pure bilirubin obtained from human gall stones, (b) bilirubin C. P., Kahlbaum, source of bile unknown, and (c) pure crystalline dog bilirubin. A table has been constructed so that knowing the colorimeter reading and the amount of bile in cubic centimeters, the bile pigment can be read off directly.

Table I (Dog 16-6) shows the output of bile and bile pigments by a dog in good condition on mixed diet plus cooked liver. The bile

secretion varies in six hours from a minimum of 42 cc. to a maximum of 69 cc. and the bile pigments from 29 mgm. to 42 mgm. The two hour periods are seen to vary greatly, but there is no relation to food which is always given at the end of the first two hours. Bile is excreted from the intestine and stercobilin is absent. There are only traces of bile pigment in the urine. This bull dog was very fat when operated upon, and the initial loss of weight during the first month is in part due to this fact. It is noted that bile fistula dogs in the best possible

TABLE I
Normal dog—mixed diet and liver

dog 16-6*	BILE								URINE BILE PIGMENTS TOTAL, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
September 16.....	17	16	19	52	7.4	9.8	16.6	33.8	trace	pounds 36.5	R.B.C. 5,632,000; Hb. 87 per cent; W.B.C. 7,600.
September 17.....	24	20	16	60	11.7	10.5	6.7	28.9	0	36.3	Stools clay colored; no stercobilin.
September 18.....				62				32.1	0	36.0	
September 20.....	16	18	21	55	11.2	15.8	9.7	36.7	0	35.5	
September 21.....	24	24	18	66	15.4	14.0	13.0	42.4	trace	36.0	
September 22.....	23	21	18	62	14.1	13.1	13.4	40.6	0	35.5	
September 23.....	20	22	27	69	12.5	11.6	12.2	36.3	+	35.8	Stools clay colored; no stercobilin.
September 24.....	13	25	19	57	6.4	12.7	11.4	30.5	+	35.5	
September 25.....				42				33.0	trace	34.5	
September 27.....	23	18	14	55	13.5	8.3	8.1	29.9	+	34.0	September 30. Hb. 98 per cent.
Average				58				34.4			

* Bile fistula operation September 2, 1915. Usual mixed diet plus 150 grams boiled sheep liver with morning meal.

condition will carry but very little subcutaneous fat. If the dog is lean and muscular at time of operation, it will lose only a little weight, but if very fat, the dog will lose several pounds after operation in spite of any care and apparent good health and appetite.

Table II (Dog 16-6) shows the same dog as Table I two months later when in apparently excellent condition. The dog was fed a mixed diet plus cooked liver and fresh dog bile. It is to be noted that the bile volume is much greater than in Table I (average per six hours—58 cc.) as compared with the average of 96 cc. We believe this chola-

gogue action is wholly due to the fresh bile feeding. On the other hand the bile pigments are *lower* (average 28.6 mgm. per six hours) as compared with Table I (average 34.4 mgm. per six hours). This drop in bile pigment output we do not believe is due to loss in weight, as the dog seemed quite normal and had a good appetite. We will discuss this point more in detail later.

The animal seemed normal in all respects until December 5, when she vomited some food. The next day she developed muscular tremors and convulsions shortly followed by death. The intoxications which

TABLE II
Normal dog—mixed diet, liver and bile

DOG 16-6*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
November 26. . .	34	36	29	99	10.8	9.6	9.8	30.2	trace	pounds 30.8	
November 27.				102				27.6	trace	30.8	Stools clay colored; no stercobilin.
November 29.	35	35	29	99	7.9	8.6	8.5	25.0	trace	31.0	
November 30	35	26	27	88	10.1	10.5	9.5	30.1	0	30.8	
December 1.	30	31	33	94	12.2	8.9	9.0	30.1	0	30.3	Hemoglobin 10% per cent; beef heart diet.
Average				96				28.6			

* Bile fistula operation September 2, 1915. Death, intoxication December 6, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal and 40 cc. fresh dog bile mixed with morning meal.

develop in consequence of long standing bile fistulas are of great interest but cannot be discussed at this time.

Autopsy in general is negative. Kidneys and other organs are normal. Liver is practically normal; no increase in fat. There is slight increase in the brownish color of the liver cells. The bile passages are all clean and pale throughout, even at the lower part of the fistula in contact with the drainage tube when it is in place. The common duct and the hepatic ducts are slightly dilated and thickened. Site of section of common duct is obliterated by dense scar tissue. The duodenal papilla is normal, and contains only mucus and no bile. Bile is completely excluded from the intestine.

Table III (Dog 15-22) shows the initial loss of weight following a bile fistula from 30.8 pounds to 24.5 pounds due to the fact that the dog was quite fat when operated upon. The bile flow varies from 66 cc. to 97 cc. per six hours (average 76 cc.), and the bile pigments from 20 mgm. to 32 mgm. per six hours (average 25.5 mgm.), but the maxima and the minima for bile flow and bile pigments do not coincide in any way. Bile pigments are constantly present in small amounts in the urine. The usual hourly variations in secretion are noted. Sterco-

TABLE III
Normal dog—mixed diet and liver

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									mgm.	pounds	
April 21.	33	30	34	97	9.6	9.5	7.8	26.9	0.25	27.3	April 20, R.B.C. 5,952,- 000; Hb. 94 per cent; W.B.C. 8,000.
April 22.	25	24	26	75	7.1	9.1	11.9	28.1	+	27.5	
April 23.	25	26	26	77	8.8	11.1	11.9	31.8	+	27.0	Stools clay colored; no stercobilin.
April 26.	27	21	23	71	7.2	8.0	10.1	25.3	0.3	25.5	R.B.C. 5,824,000; Hb. 96 per cent.
April 27.	15	26	25	66	3.6	8.3	7.9	19.8	0.2	25.0	
April 28.	21	22	15	68	6.5	7.4	7.0	20.9	0.3	24.5	
Average.				76				25.5			

* Bile fistula operation April 2, 1915, weight 30.8 pounds. Usual mixed diet plus 100 grams boiled sheep liver with morning meal.

bilin is absent, and bile presumably absent from the intestine, which is to be contrasted with Table IV in the same dog.

Table IV (Dog 15-22) is of considerable interest because the dog's stools now contain some stercobilin. This period is about four months after Table III observations of April. In May it was noted that the dog had gained weight up to his original weight of 30.8 pounds, and was in unusual condition for a bile fistula dog on a liver and mixed diet. Stercobilin was found in the feces constantly from this time on, and we were forced to the conclusion that by means of a small fistulous tract (as observed previously in the dogs at autopsy) a small amount

of bile escaped into the duodenum when the pressure in the common duct was elevated.

From great numbers of observations on this dog and others with no bile escaping into the duodenum at any time, we are convinced that the collections from this dog, 15-22, represent his total output during the periods of six hours, we know that such fistulous tracts as this dog must have are very small and tortuous, and permit of only small amount of bile escaping even under high pressure. His output (Table III), before this fistulous tract into the duodenum was established, is practi-

TABLE IV
Normal dog—mixed diet and liver—bile in intestine

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
August 17	18	16	18	52	10.2	7.9	8.6	26.7	0	33.3	Hemoglobin 129 percent
August 18.....	14	11	18	43	9.1	9.0	9.7	27.8	0	33.3	
August 19	17	14	16	47	9.2	8.5	8.8	26.5	0	33.3	
August 20...	16	17	15	48	9.4	7.8	8.6	25.8	0	33.3	Stools contain stercobilin
September 15....	27	17	25	69	8.3	6.3	6.8	21.4	0	33.	
September 16.....	23	17	28	68	9.1	8.2	10.7	28.0	0	32.8	
September 17....	18	20	26	64	8.1	9.7	11.1	28.9	0	33	R.B.C. 7,848,000; Hb. 125 per cent; W.B.C. 9,600
September 18				56				26.7	0	33	
Average				56				26.5			

* Bile fistula operation April 2, 1915. Usual mixed diet plus 200 grams cooked sheep liver with morning meal.

cally identical with that in Table IV, the after period. His output of bile compares exactly with that of similar dogs with complete fistulae, shows the same fluctuations and the same average output. When the dog is put up in harness with a tube draining his gall bladder, it seems almost certain that all the bile escapes through this tube as the flow takes place by gravity. When the dog is curled up in his cage with his bile fistula partially closed and compressed by his posture, much bile escapes into the cage, and a small amount escapes into the duodenum along the path of the resected and ligated common duct. This bile is sufficient to maintain the dog in normal condition.

Table V (Dog 15-22) resembles in general Table IV, and shows the same constantly high hemoglobin curve. There is a little loss of weight, but the dog is in perfect condition. This table covers a period of five days *following* a period of four days of sexual intercourse and excitement, during which period of over excitement the bile output was double normal. We have more data on this point which we will report later. These data are given to explain in part at least the abnormally high initial curve of pigment excretion in this dog with the progressive fall during the week (Table V).

TABLE V
Normal dog—mixed diet

DOG 15-22*	BILE								URINE BILE PIG- MENT TOTAL— SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1915										grams	
November 26....	13	18	19	50	12.5	15	14.5	42.0	0	31.0	November 11, hemo- globin 121 per cent.
November 27....				39				36.8	0	31.3	Stools contain stercobilin
November 29....	19	22	20	61	7.0	16.5	12.9	36.4	0	31.5	
November 30....	23	23	23	69	6.2	7.4	8.8	22.4	trace	31.3	Hemoglobin 124 per cent
December 1.....	21	22	25	68	9.4	10.1	10.7	30.2	0	31.3	
Average.....				57				33.6			

* Bile fistula operation April 2, 1915. Mixed diet of meat, bones, bread, and no liver.

Table VI (Dog 16-5) is a good example of the fluctuation of the bile pigment curve which may be found following a bile fistula operation associated with icterus of a mild but definite degree. For some reason drainage was not good after the operation, and for many days after normal drainage was established we see the wide fluctuation in output from a minimum of 18 mgm. to a maximum of 58 mgm. The weight remains constant, and the dog is active and hungry. Any procedure taken up during any such period could give no information, and would only lead to confusion. We believe the icterus is in part responsible for these great fluctuations in bile pigment excretion. Gradually the curve of pigment excretion becomes more uniform as seen in the next Table VII.

Table VII (Dog 16-5) again shows the cholagogue action of bile given with the food (compare Table VI), and gradually the bile pigment excretion becomes fairly uniform.

One notes on two days (October 1 and 5) that the second period of collection shows a very small excretion. This is not due to any error in collection, as the dog was under constant observation, and we are not prepared to give a satisfactory explanation. It has been noted

TABLE VI
Post-operative icterus—mixed diet and liver

dog 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
September 15.....	22	15	15	52	17.8	15.3	16.9	50.0	++++	pounds 30.5	R.B.C. 5, 472,000; Hb. 89 per cent; W.B.C. 8,200; definite jaundice
September 16.....	20	15	20	55	21.7	19.7	17.2	58.6	++++	31.0	Stools contain no stercobilin.
September 17.....	22	15	24	61	13.9	12.2	21.1	47.2	+++	31.3	
September 18.....				47				34.9	++	31.5	
September 20.....	19	9	11	39	22.5	16.4	14.5	53.4	+	30.5	
September 21.....	24	8	21	53	12.7	4.2	14.2	31.1	+	29.5	Slight jaundice
September 22.....	13	8	14	35	4.3	4.0	9.5	17.8	+	29.5	
September 23.....	7	17	24	48	2.5	7.8	10.8	21.1	++	29.0	Stools contain no stercobilin.
September 24.....	20	17	33	70	3.1	5.0	11.9	20.0	++	29.0	
September 25.....				46				12.4	++	29.3	Slight jaundice.
September 27.....	14	21	19	54	7.0	10.6	9.2	26.8	++	29.8	
September 28.....	23	15	16	54	6.5	6.1	6.3	18.9	++	30.0	
Average.....				53				32.7			

* Bile fistula operation September 1, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

that soon after operation (two weeks) the introduction of the rubber tube may be followed by an hour or more of almost complete cessation of the bile flow. This may be due to a nervous reflex resulting from the slight pain caused by the catheter in the recent fistula or due to the excitement of the novel surroundings and an unfamiliar procedure. We may be able to give a satisfactory explanation as more observations accumulate, but at present we believe such periods of inhibition of flow may be due in part to some nervous reflex.

TABLE VII

Slight icterus—mixed diet and fresh bile

DOG 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
September 29	27	17	23	67	8.3	4.0	9.5	21.8	++	30.3	Stools contain no stercobilin.
September 30.....	15	24	20	59	6.2	5.5	7.4	19.1	++	30.5	Slight jaundice
October 1..	34	8	36	78	10.7	2.9	11.3	24.9	++	30.5	
October 2 ..				62				29.3	++	30.5	
October 4	27	16	20	63	9.1	6.5	8	24.4	++	30.8	
October 5	31	10	25	66	12.4	4.3	12.4	29.1	++	31.0	
October 6..	26	21	25	72	9.8	10.3	10.1	30.2	++	30.8	October 22—R.B.C. 6,360,000; Hb. 93 per cent; W.B.C. 13,400.
Average				67				25.5			

* Bile fistula operation September 1, 1915. Mixed diet plus 30 cc. fresh dog bile with morning meal and 60 cc. fresh dog bile with evening meal.

TABLE VIII

Poor condition—duodenal ulcer

Dog 15-16*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									<i>mg.</i>	<i>pounds</i>	
April 14.	55	27	44	126	17.3	7.7	10.9	35.9	0	42	April 5, R.B.C. 5,776,000; Hb. 90 per cent; W.B.C. 7,200.
April 15.	41	42	24	107	9.2	10.3	6.6	26.1	0	41.5	Stools contain no ster- cobilin.
April 16.	35	53	19	107	8.5	15.5	6.8	30.8	0	41.0	
April 19.	22	27	30	79	18	23.1	28.5	69.6	0.7	39.5	Slight jaundice.
April 20.	25	27	23	75	14.6	16.9	19.0	50.5	0.5	39.3	R.B.C. 5,408,000; W.B.C. 7,000. Stools contain no stercobilin.
Average.								42.6			

* Bile fistula operation March 24, 1915. Death April 23. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

Table VIII (Dog 15-16) shows a good example of a dog which lost ground steadily after the operation in spite of careful feeding and every attention. His bile output is fairly constant, but the bile pigment secretion varies from 26 to 70 mgm. per six hours. He developed a slight grade of icterus and lost all appetite. He was sacrificed because of poor general condition.

Autopsy gave the following information. Liver seemed normal in gross, but under the microscope showed a little fatty degeneration. Bile passages all normal, and bile completely excluded from duodenum. Large duodenal ulcer (1.5 by 1 cm.) about 3 cm. below pylorus extending deep into muscular coats gives no evidence of hemorrhage. Prostate is huge. Microscope shows the common cyst adenoma of the gland. The dog was quite old. The old age and the duodenal ulcer may be in part responsible for the rapid failure of this dog after the bile fistula operation. However, he showed some signs of the characteristic intoxication which so frequently carries off the animals.

DISCUSSION

A study of the above tables will emphasize the point which Stadelmann insists upon with so much justice, namely, the normal flow of bile from a bile fistula dog is subject to wide fluctuations, which cannot at present be explained. This includes fluctuation in the total output and the bile pigment secretion, but it is to be noted that these maxima do not coincide. A low output of bile may contain a high total bile pigment content and vice versa. Stadelmann points out the fact that bile pigment and bile salt secretion have no relation, and he argues from this that the function of the liver cell is double and quite independent in these two respects. With this point in mind, it is obvious that one must be very careful in analysis of any observations on bile secretion. Experiments must be repeated again and again, and a seemingly unnecessary number of control observations must be recorded before and after the actual experiment. It is seen in the above tables that with care and proper food a dog may have a fairly long period of relatively uniform secretion. Such periods are most favorable for experimental work.

We realize that criticism may be offered against our six hour period of bile collection. Many of the recorded experiments of other workers show twenty-four hour collections or even periods of several days during which time the dog is suspended comfortably in a sling. Others

use ten or twelve hour periods for several consecutive days. After some observations, it seemed best in the long run to make shorter daily collections over many weeks or months, obtaining the bile every day during the same hours, the dog having a constant daily routine. The dog can live a pretty normal existence, and, most important of all, can maintain a pretty constant bodily condition for a long period of time. Such bile collections perhaps represent more nearly the normal flow as it occurs in a normal dog under laboratory conditions.

It will be seen after a careful study of the above tables that these bile fistula dogs on the mixed diet have a pretty uniform *average* excretion of bile pigments -about 1 mgm. per pound body weight per six hours. Our animals are even more constant in this respect than those studied by Stadelmann and his co-workers, but our average is practically identical with their published reports. This indicates that the method used by Stadelmann (spectrophotometric) gives the same general results as our method. The figures given by Brugsch, Kawashima and Yoshimoto are about three times as high as those just reviewed, and can scarcely be accepted as correct.

SUMMARY

Our experiences with bile fistula dogs indicate that bile is essential for the life of the animal on a mixed diet of meat, bones, and bread. If bile is *wholly* excluded from the intestinal tract, the dog loses ground steadily, shows intestinal disorders accompanied by blood in the feces, and usually within a month dies with peculiar symptoms of intoxication.

Fresh pig's bile given by stomach tube and dried ox bile given in capsules will sometimes improve the condition but not to any notable degree.

Fresh dog's bile mixed with the food will sometimes give good results if the dog will eat the mixture. Given by stomach tube the results are not favorable.

Cooked liver added to a mixed diet usually keeps the dog in good healthy condition for a long period of time. At present we are not prepared to explain this observation, but the fact may have some clinical application.

Under very uniform conditions the bile pigment excretion may form a pretty uniform curve, and experimental variations under such circumstances will have some value. The usual average bile pigment excretion amounts to one milligram per pound body weight per six hours,

but there are some individual variations and considerable daily and hourly variation.

When a dog is not in good condition and perhaps is suffering from icterus or cachexia or both, we may see very great fluctuations in the bile pigment excretion curve. Experimental observations under such conditions are worse than useless, and can lead to no conclusions of value.

BIBLIOGRAPHY

- (1) ALBU: Berlin klin. Wochenschr., 1900, xxxvii, 866 and 892.
- (2) BRUGSCH AND YOSHIMOTO: Zeit. f. Exper. Path. u. Therap., 1910-1911, viii, 639.
- (3) BRUGSCH AND KAWASHIMA: Zeitschr. f. Exp. Path. u. Therap. 1910-1911, viii, 645.
- (4) HOOPER AND WHIPPLE: Journ. Exp. Med., 1916, xxiii, 137.
- (5) McNEE: Jour. Path. and Bact., 1913-1914, xviii, 325.
- (6) RANSOM: Brit. Med. Journ. 1896, ii, 897.
- (7) STADELMANN: Der Icterus und seine verschiedenen Formen. Stuttgart, 1891.
- (8) STADELMANN: Therap. Monatsschr., 1891, v, 512 and 562.
- (9) STADELMANN: Zeitschr. f. Biol., 1897, n. f., xvi, 1.
- (10) WERTHEIMER: Arch. de Physiol. norm. et path., 1892, 5, s, iv, 577.
- (11) WHIPPLE AND HOOPER: Journ. Exper. Med., 1913, xvii, 593.
- (12) WHIPPLE AND HOOPER: Journ. Exper. Med., 1913, xvii, 612.
- (13) WHIPPLE, PEIGHTAL, CLARK: Johns Hopkins Hosp. Bull., 1913, xxiv, 343.
- (14) WHIPPLE, MASON, PEIGHTAL: Johns Hopkins Hosp. Bull., 1913, xxiv, 207.

BILE PIGMENT METABOLISM

II. BILE PIGMENT OUTPUT INFLUENCED BY DIET

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The preceding paper shows the normal bile pigment secretion in dogs with permanent bile fistulae. The normal fluctuations are obvious, and must be taken into consideration in the analysis of any experiments. This paper gives observations which make it clear that the curve of bile pigment secretion can be depressed below normal by a meat diet, and can be raised much above normal by a diet rich in carbohydrates.

It seems that this observation must dispose of the commonly accepted belief concerning the origin of bile pigments; namely, that they can be formed only by the breaking down of red blood cells. Can one assume that a carbohydrate diet will cause the dissolution of a small army of red blood cells to explain the fact that the output of bile pigment may be almost doubled in a sharp transition from a meat diet to a diet rich in carbohydrates? This seems improbable to say the least.

Methods and operative procedures, care of animals and collection of bile have all been described in detail in the preceding paper. We can not give all our experimental data on this diet question, but there is complete agreement in the fundamental observation that a dog will show a low bile pigment output on a meat diet and a definite increase (sometimes 100 per cent increase) on a diet rich in carbohydrates. The two following experiments are given in considerable detail, because the two dogs were under observation for a long period of time in perfect health, showed a constant hemoglobin curve, and only traces of bile pigment in the urine at times. The observations on a single large dose of carbohydrate can be multiplied indefinitely, but they confirm the more difficult prolonged diet period experiments extending over weeks.

The Tables A, B, and C show that sugar by mouth will cause an increase in bile pigment output in a dog on a meat diet. There is a slight decrease in total bile flow during this period.

TABLE A
Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
October 21.....	24	23	26		10.0	1.4	13.2		0	32.0	Lean beef diet plus 200 grams cooked liver R. B. C. 7; 640,000; Hb. 123 per cent; W. B. C. 8600. Stools contain stercobilin.
October 22.....	20	13*	12	22	9.2	14.3*	17.7	17.4	0	31.5	
October 23.....	24				13.7				0	31.8	
October 25.....	22	22	23		10.9	9.7	10.9		trace	32.0	
October 26.....	17	18	16		8.0	9.3	7.9		0	31.8	

* Given 80 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

TABLE B
Dextrose feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
June 7	24	25	22		4.3	4.5	5.3		trace	31.5	Lean beef diet plus 200 grams cooked liver Stools contain stercobilin
June 8	19	18*	11	14	4.6	6.7*	7.6	9.1	trace	31.5	
June 9	11	11	12		4.5	5.2	7.6		trace	30.5	
June 10	23	24	26		4.1	4.8	4.7		trace	30.8	

* Given 200 grams dextrose in 400 cc. water by stomach tube at beginning of third hour.

TABLE C
Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
October 14.....	22	24	22		5.9	10.0	11.1		0	32.5	Lean beef diet plus 200 grams cooked liver
October 15.....	28	11*	15	16	5.6	8.5*	14.2	20.9	0	33.0	
October 16.....	22				11.9				0	32.8	
October 18.....	31	26	20		10.3	8.0	8.5		0	32.0	
October 19.....	22	24	27		9.2	8.5	10.1		0	32.0	

* Given 60 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

Tables D and E show that dextrose given intravenously will also cause a rise in the bile pigment curve of excretion, one experiment on a mixed diet and the second on beef heart diet. These experiments

TABLE D
Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS			
	Amount in cubic centimeters				Bile pigments in milligrams									
	1-2 hrs	3-4 hrs	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs	5-6 hrs.	7-8 hrs						
May 18...	19	21	18		6	8	7	1	7	0	trace	30.0	Mixed diet plus 200 grams cooked liver	
May 19...	19	23*	34	27	3	9	6	2*	12	1	10	0	30.5	Stools contain stercocobilin. May 22, R.B.C. 5,696,000: Hb. 91 per cent.
May 20...	18	20	21		3	6	5	4	7	1	+	31.0		
May 21...	24	24	24		5	4	7	0	6	9	trace	31.0		

* 50 grams dextrose in 1000 cc. 0.7 per cent salt solution given intravenously at beginning of third hour.

TABLE E
Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
										Beef heart diet	
										ounces	
December 13 . . .	32	23	27		7.2	6.7	8.5		0	31	December 11, hemo- globin 131 per cent.
December 14	33	32	23		8.0	9.4	8.6		0	31.5	Stools contain stercobilin.
December 15.....	31	29*	16	15	7.5	11.5*	10.5	8.1	0	31.8	
December 16.....	23	22	23		14.1	14.5	12.2		0	31.0	
December 17.....	26				12.7				0	30.5	

* 600 cc. 6 per cent dextrose given intravenously at the beginning of the third hour.

show little decrease in bile flow, especially in Table D, where the larger amount of fluid (1000 cc.) was given intravenously. Such variations, however, come within physiological limits, and no importance is to be attached to them.

Table F shows in a convincing way that a meat diet gives a much lower output than a diet rich in carbohydrates. The meat diet period of six days shows an average output of 29.1 mgm. bile pigment, which is not very low, as can be seen in the after-period of eleven days with an average of 25.6 mgm. (Table H). This same dog at other times on a meat diet has gone as low as 16 mgm. bile pigment output average of six days.

TABLE F

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1915										<i>p und</i>	Boiled lean meat diet
October 25.....	22	22	23	67	10.9	9.7	10.9	31.5	trace	32	October 22, R.B.C. 7,640,- 000 Hb. 123 per cent.
October 26.....	17	18	16	51	8.0	9.3	7.9	25.2	0	31.8	
October 27.....	20	18	19	67	6.1	9.8	8.1	24.0	trace	31.8	
October 28.....	18	17	21	56	9.0	9.6	12.0	30.6	trace	31.5	
October 29.....	20	21	26	67	9.5	11.3	11.1	31.9	trace	31.8	
October 30.....				56				31.5	+	31.0	Stools contain stercobilin.
Average ..				59				29.1			

Change from lean meat diet to bread, milk and bones

November 1.....	15	16	18	49	13.5	15.1	16	44.6	trace	31.5	Stools contain stercobilin. October 25, Hb. 123 per cent.
November 3.....	16	18	20	54	14.4	17.1	16.7	48.2	trace	31.8	
November 4.....	17	23	20	60	16.0	19.1	16.3	51.4	trace	31.8	
November 5.....	15	20	19	54	13.5	13.5	13.3	40.3	trace	32.0	
November 6.....				45				41.5	trace	31.8	
Average				52				45.2			

The sharp transition to the diet of bread, milk and bones gives a great rise to an average of 45.2 mgm. bile pigment, an increase of over 50 per cent bile pigment elimination. There is a trifling decrease in average bile flow from 59 cc. to 52 cc.

Table G shows that a continuation of the bread, milk, and bone diet does not maintain the bile pigment output at the maximum of 45.2 mgm. of the previous week, but the average is 39.7 mgm. bile pigment. This same fact is noted in another dog (Table K), and there is

a tendency for the high bile pigment curve of a carbohydrate diet to approach the mean curve of a mixed diet. Also there is the same tendency for the low bile pigment curve of a meat diet to approach the mean curve of a mixed diet. This applies particularly to dogs kept for several weeks on a meat diet or a carbohydrate diet. The meat diet dog may show periods of rise in bile pigment output close to the mean curve of a

TABLE G

Bile pigment secretion on carbohydrate diet and fat

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
November 8 . . .	17	20	24	61	11.9	13.6	12.7	38.2	0	31.3	Diet of bread, milk, and bones Hemoglobin 121 percent
November 9	19	23	21	63	13.3	14.2	12.8	40.3	0	31.3	
November 10 . . .	21	21	25	72	8.0	9.2	12.4	29.6	trace	31.3	
November 11	28	26	25	79	11.1	11.4	10.5	33.0	trace	31.5	
November 12	16	23	22	61	12.6	13.2	12.6	38.4	trace	31.3	
November 13				55				42.5	trace	31.3	
November 15 . . .	14	23	21	58	14.5	13.2	14.4	42.1	0	31.0	
November 16 . . .	18	23	20	61	15.0	18.2	12.6	45.8	trace	31.5	
November 17	20	19	23	62	16.7	15.8	15.2	47.7	trace	31.3	
Average				64				39.7			

Same diet plus 100 cc. cotton seed oil with morning and evening feeding

November 18 . . .	13	18	19	50	15.2	16.6	16.2	48.0	0	31.5	November 30, hemo- globin 124 per cent.
November 19	24	23	19	66	10.8	11.1	9.6	31.5	0	31.8	
November 20				45				27.8	0	31.5	
Average				60				35.7			

mixed diet. The diet rich in carbohydrate may give a very high initial curve (perhaps double normal), which is apt to fall during succeeding weeks, but always remains somewhat above the mean curve.

Cotton seed oil fed with this bread, milk, bone diet is associated with a slight drop in bile pigment elimination, but another dog (Table K) gives negative results. We hope to do much more work with various fats and lipoids.

Table H shows the after-period on a beef heart diet with an average output of 25.6 mgm. bile pigment. The flow of bile is somewhat increased on this diet from 60 cc. to 83 cc. per six hours.

The importance of these observations (Tables F, G, H) lies in part in the fact that this dog was under constant observation with daily collections of bile for a period of about eight weeks in perfect health with uniform hemoglobin curve. The deduction to be drawn from changes in diet under such uniform conditions will be of value, and the sequence of events is not to be lost sight of: (1) Meat diet and low

TABLE H
Bile pigment secretion on beef heart diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
December 2.....	39	27	28	94	7.8	10.3	9.9	28.0	trace	points 32.0	November 30, hemo- globin 124 per cent.
December 3.....	25	26	28	79	8.3	8.2	8.1	24.6	0	32.2	
December 4.....				79				24.9	0	31.3	Stools contain stercobilin
December 6.....	24	21	22	67	9.5	9.9	7.7	27.1	0	31.0	
December 7.....	27	28	31	86	8.3	10.1	12.3	30.7	0	31.5	
December 8.....	34	33	24	91	5.4	10.4	8.3	24.1	0	31.8	
December 9.....	38	28	26	92	5.1	6.7	7.6	19.4	0	31.8	Hemoglobin 131 percent
December 10.....	33	35	24	92	7.3	10.9	8.5	26.7	0	31.8	
December 11.....				64				28.2	0	31.5	
December 13.....	32	23	27	82	7.2	6.7	8.5	22.4	0	31.0	Stools contain stercobilin.
December 14.....	33	32	23	88	8.0	9.4	8.6	26.0	0	31.5	
Average.....				83				25.6			

bile pigment elimination: (2) bread, milk, bone diet and very high bile pigment curve: (3) second period of bread, milk, bone diet and constant high pigment curve; (4) same diet with oil shows slight fall in bile pigment curve: (5) end period of beef heart diet with low bile pigment elimination (see table M).

Table J confirms the observations in Table F, but the change here is not so striking. On a meat diet the dog put out 27.7 mgm. bile pigment, and on a bread, milk, bone diet eliminated 37.5 mgm. bile pigment. It is to be noted that this dog was losing weight during this

period, but seemed in good health, and the hemoglobin curve was uniform.

Table K shows a slight fall during the second week of carbohydrate feeding, but it remains above the meat diet period, and shows no depression as the result of adding cotton seed oil to the same diet.

TABLE J

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
October 25.....	30	27	22	79	10.8	10.3	9.7	30.2	0	34.5	Boiled lean meat diet plus 200 grams boiled liver
October 26.....	26	25	27	78	7.6	7.7	8.9	24.2	0	34.0	
October 27.....	25	24	23	72	7.3	7.9	9.6	24.8	0	33.8	Stools contain no stercobilin.
October 28.....	21	20	21	62	10.0	8.3	10.6	28.9	trace	33.3	
October 29.....	25	23	16	64	10.1	10.4	9.4	29.9	0	33.0	
October 30.....				54				27.8	0	32.5	Hemoglobin 103 percent
Average.....				68				27.7			

Change from lean meat diet to bread, milk and bones

November 1..	16	18	20	54	10.8	13.8	11.9	36.5	0	31.5	October 30, hemoglobin 103 per cent.
November 3..	17	18	20	55	13.4	11.3	15.0	39.7	0	31.5	
November 4..	22	20	22	64	12.9	13.2	13.1	39.2	0	31.0	
November 5..				54				37.8	trace	30.8	
November 6..				66				34.1	trace	30.8	Stool contain no stercor- bilin.
Average..				59				37.5			

The after-period (Table L) is unsatisfactory because rather too short as a result of the death of the dog. She was apparently in perfect health December 4, and it is proper to include this reading in the table. The next day she refused food, and vomited once, but did not appear sick. On December 6 she died with peculiar symptoms of intoxication, and the autopsy abstract is given in the preceding paper (following Table II—Dog 16-6).

TABLE K

Bile pigment secretion on carbohydrate diet and fat

DOG, 16-5	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
November 15....	23	31	29	83	9.3	10.3	10.3	29.9	trace	31.5	Bread, milk and bone diet plus 200 grams boiled liver
November 16....	30	31	29	90	10.0	11.7	10.3	32.0	trace	32.0	
November 17.....	22	28	35	85	9.7	13.0	8.6	31.3	trace	32.3	Stools contain no ster- cobilin.
Average.....				86				31.1			

Same diet plus 100 cc. cotton seed oil with morning and evening feeding

November 18.....	28	27	30	85	10.5	8.4	8.8	27.7	trace	32.5	December 1, hemoglobin 108 per cent.
November 19.....	35	33	35	103	14.8	8.8	8.7	32.3	trace	32.5	
November 20.....				112				30.2	0	32.5	
November 22.....	18	28	34	80	11.9	12.4	12.1	36.4	trace	31.0	
November 23.....	29	22	34	85	10.3	10.0	9.8	30.1	trace	31.8	
November 24.....	35	23	31	89	11.0	12.2	10.5	33.7	trace	31.3	
Average.....				92				31.7			

TABLE L

Bile pigment secretion on beef heart diet

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in millimeters						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
December 1	30	31	34	95	12.2	9.0	9.0	30.2	0	30.3	Boiled beef heart diet plus 200 grams boiled liver
December 2	35	39	30	104	7.8	9.9	8.1	25.8	0	30.0	
December 3.....	36	35	31	102	7.3	7.8	6.9	22.0	0	30.8	
December 4.....				112				17.6	0	31.3	
Average				103				23.9			

TABLE M

Average readings from above tables

DOG	LEAN MEAT DIET		BREAD, MILK, BONE MEAT DIET		BREAD, MILK, BONE MEAT DIET WITH OIL		BREAD, MILK, BONE BEEF HEART DIET		REMARKS
	Bile	Bile pigment	Bile	Bile pigment	Bile	Bile pigment	Bile pigment	Bile pigment	
	cc.		cc.		cc.		cc.		
Dog 15-22	59	29.1	52	45.2					
Average per 6 hours			64	39.7	60	35.7	83	25.6	Weight, 32 pounds
Dog 16-6	68	27.7	59	37.5					
Average per six hours			86	31.1	92	31.7	103	23.9	Weight, 31 pounds.

This table gives the average readings of bile and bile pigment output during the various diet periods. They are arranged in the same sequence in which the experiments were performed. For study of the details one must refer to the other tables which are of greater interest.

DISCUSSION

From the above tabulated experiments it is clear that a diet rich in carbohydrates or sugar by mouth or dextrose intravenously will increase the secretion of bile pigments. We believe the data are sufficient to establish this as a fact, but how may we explain this increase in bile pigments following the administration of a carbohydrate? There are numerous possibilities which must be tried out by various experimental procedures, and we will merely mention a few of them.

Sugar feeding at once suggests a storing of glycogen in the liver, and its deposition in the liver cell may accelerate the metabolic activity of the cell or stimulate it to produce greater amounts of bile pigments than under normal conditions.

We must recognize, too, that a meat diet tends to depress the bile pigment secretion below the usual level of a mixed diet. Any explanation suggested to explain the carbohydrate stimulus must also explain the protein diet depression of bile pigment output. It is possible that a meat diet in dogs represents a normal condition, and that the low curve of pigment excretion on the meat diet is the true normal excretion. The rise on a simple mixed diet of bread and meat may then be explained by the addition of the carbohydrate and the maximum output on the bread, milk, bone diet as due to a great increase in the

carbohydrate portion of the diet. It will be of considerable interest to observe the bile pigment output on a pure carbohydrate diet. This is a difficult type of experiment for a bile fistula dog, but it may give results of considerable value when carried to a successful termination.

Here it may be mentioned that we have pretty good evidence that bile or blood feeding do not greatly increase the bile pigment output. We hope to report detailed work on this important point in the near future.

When it is suspected that the liver can form bile pigments out of various materials other than hemoglobin, which is so closely related chemically to bile pigment, one first thinks of substances rich in the pyrrole nucleus. Bile pigment or blood feeding should give a great increase in bile pigment output, which is not the fact. One must go back further in the development of the body pigments and ask: where does the hemoglobin come from? A prompt answer is made that hemoglobin is formed in the red cells in the bone marrow. But it is at least possible that these cells may merely put the finishing touches on this complex substance, which may be built up in great measure in some other tissue. In other words, there may be a prehemoglobin substance manufactured somewhere in the body, perhaps in the liver, which may be fixed by the bone marrow cells, and appear as finished hemoglobin. If it can be established that the liver cells form any such substance, a long step will have been made toward the solution of this complex question of pigment metabolism.

SUMMARY

A large dose of sugar by mouth will give a constant reaction in a healthy dog with a bile fistula. It will cause a definite increase in bile pigment excretion over a period of several hours.

The same rise in the curve of bile pigment elimination follows intravenous injection of dextrose.

A mixed diet in a healthy bile fistula dog is associated with a fairly constant *mean* bile pigment elimination.

A change to a meat diet will give a depression of this average bile pigment elimination.

A change to a diet rich in carbohydrates will give a sharp rise in bile pigment output—often 30 to 100 per cent increase. Such modifications of bile pigment elimination may be carried on indefinitely with a healthy animal.

We believe established the fact that carbohydrates stimulate the excretion of bile pigments in bile fistula dogs, but a convincing explanation of this phenomenon we can not bring forward at this time. More work is required.

It seems, however, that these facts must overthrow the long accepted theory that bile pigment is formed only as a result of the disintegration of red blood cells.

It is at least possible that the liver has some constructive ability in pigment formation which can be modified by diet. It is also possible that the liver may be concerned in building up other body pigments than bilirubin—for example, hemoglobin.

STUDIES ON THE BLOOD PROTEINS.

I. THE SERUM GLOBULINS IN BACTERIAL INFECTION AND IMMUNITY.

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INTRODUCTION.

For a number of years much study has been devoted to the origin and the chemical nature of the antibodies which may develop within the organism during the course of an infection, or which may be elaborated within it by the various methods of immunization. The efforts to establish the chemical identity of antibodies have naturally been centered about a study of the possible relationship subsisting between the proteins of the blood and the immune bodies demonstrable in it by various serologic tests. A great stimulus to these investigations has come from the discovery of new methods of separating and of chemically identifying the different fractions which go to make up the blood proteins. Of these additions to our knowledge the method, introduced by the Hofmeister school, of separating the various protein constituents by fractional precipitation with different salts has, perhaps, produced the most far reaching results.

For some time it has been a well established fact that diphtheria antitoxin, for instance, is precipitable from serum by any precipitants which throw down the globulins. The early observations of Brodie (1), Seng (2), and Hiss and Atkinson (3) have been confirmed and extended by a number of later workers (4).

Considerable work has been done also to establish the chemical nature of bacterial antibodies. Probably one of the earliest contributions to this subject was made by Pfeiffer and Proskauer (5), who separated cholera immune serum into its globulin and albumin fractions, and showed that the cholera immune bodies which give rise to Pfeiffer's phenomenon are present only in the globulin fraction. This important study was later amplified by the classical experiments of Pick (6), who demonstrated conclusively that cholera and typhoid agglutinins also occur in one or another of the globulin fractions depending upon the species

of animal employed for immunization and the nature of the antibody studied. To these observations should be added those of Rodhain (7) and of Moll (8). According to the work of Rodhain, the immune bodies of antistreptococcus serum occur in the euglobulin fraction; and according to Moll, the development of precipitins following immunization with a foreign protein is also associated with a rise in the serum globulins of the immunized animal.

The above observations have been variously interpreted by different investigators. Whereas some workers strongly incline to the view that the antibodies in question are a form of blood globulin, others entertain the possibility that the antibody, by analogy with bacterial poisons, enzymes, and similar bodies, is mechanically carried down by the precipitate of globulin.

We believed that the only satisfactory method of procuring reliable data on the globulin-antibody problem was to make quantitative estimations of the immune bodies and of the blood proteins, not at random periods during the experiment, but at frequent and well timed intervals during the process of immunization. In this way alone is it possible to determine whether an increase in the antibodies and in the globulins parallels one another, or whether either the globulin content or the concentration of immune bodies may increase independently of one another.

Methods.

Healthy Belgian hares kept under constant conditions of diet and activity were used for all the experiments. The animals were fed once daily, the diet consisting of alfalfa hay, toasted bread, oats, and at times small amounts of cabbage. All were allowed a liberal amount of water.

Obtaining Blood.—Specimens of blood, varying in amounts from 5 to 7 cc. were obtained from fresh incisions made in the ear vein. In all instances the use of local applications of xylol or other substances that might cause stasis was avoided. At times the presence of a low blood pressure made bleeding difficult, but in such cases a free flow of blood was obtained by suspending the rabbit by its hind legs. The blood obtained in small sterile tubes was immediately centrifugalized in order to obtain a clear serum. Separation of the serum from the clot was effected as soon as possible, for as will be shown later, serum allowed to remain in contact with the clot is not suitable for accurate determinations of the proteins. The specimens before use were kept in sterile, stoppered vials in the refrigerator.

Tests for Agglutination.—The antigen used for the agglutination tests consisted of a 24 hour carbolized or formalinized Liebig's or rabbit broth cul-

ture, which had been properly controlled by tests with immune sera of known antibody content. To a series of tubes containing the clear, untreated serum in amounts ranging from 0.1 cc. to 0.00005 cc. was added 1 cc. of antigen. After incubating the mixtures for 2 hours at 37° C. or at room temperature for 12 hours readings were made. The final readings were always made after the lapse of 12 or 14 hours. The highest dilution of the serum in which complete agglutination occurred was taken to represent the agglutination titer of that serum, and only these readings are recorded in the tables.

Tests for Complement Fixation. Antigens.—Cultures of the various organisms grown upon lemco broth¹ for 18 to 20 hours² were found to be most suitable for this purpose. They were killed by heating for $\frac{1}{2}$ hour at 60°C., and preserved with 0.5 per cent carbolic acid and 1 per cent glycerol. When kept in the refrigerator and protected from the light such antigens may be ready for use after a period of 2 months. Some, however, may become anticomplementary after a period of 4 weeks, and these must be discarded.

The dose of antigen employed was four times the antigenic unit as determined by preliminary titrations of the antigen with a standard amount of immune serum; either 0.1 or 0.2 cc. This dose was at least one-quarter to one-fifth of the anticomplementary unit as determined by repeated titrations. The range of the specific antigenic properties of each antigen was further controlled by tests with sera of known antibody content.

Sera.—Dilutions of inactivated serum (62°C. for 30 minutes) in descending doses from 0.2 to 0.003 cc. were used. In selecting such dilutions it frequently occurred that the gradations were not well chosen. This made it difficult to express the results absolutely in terms of the highest dilution of serum which gave definite fixation. For this reason the signs $><$ are employed. Thus the notation > 0.005 indicates that complete fixation of the complement would probably have occurred in a serum dilution of 0.004 or 0.003 cc., since in a dilution of 0.002 cc. of the serum only 50 per cent fixation was obtained. In the tables only those dilutions of the serum are recorded which with the proper dose of the antigen caused a complete fixation of the complement.

Hemolytic System.—The anti-sheep hemolytic system was used. Complement was furnished by the pooled sera of several guinea pigs, and was employed in a dosage of 0.05 cc. of a dilution of 1 to 4 in salt solution. The red corpuscles were used in 1 per cent suspension. The hemolytic unit was determined by a preliminary test using 0.05 cc. of complement and 0.5 cc. of a 1 per cent suspension of fresh sheep cells. In the titrations of the antigens as well as for the actual complement fixation tests two hemolytic units (about 0.2 to 0.1 cc. of a dilution of 1 to 100 in saline solution) were employed.

¹ Eyre, J. W. H., *The Elements of Bacteriological Technique; a Laboratory Guide*, Philadelphia and London, 2nd edition, 1913, 163.

² Cultures less than 18 hours old are frequently inactive, and those older than 24 hours may be anticomplementary in doses of 0.5 cc.

Technique.—Preliminary tests were first made to rule out any anticomplementary activity of the different antigens used. These were carried out as follows: To each of a series of tubes containing decreasing doses of antigen diluted with 1.5 cc. of isotonic salt solution was added 0.05 cc. of complement in a dilution of 1 to 4. The tubes were then incubated at 37° C. either in a water bath for $\frac{1}{2}$ hour or in an incubator for 1 hour. To each tube was then added a previously prepared mixture of two units of hemolysin and 0.5 cc. of the corpuscle suspension. After mixing and incubating for 1 or 2 hours, sedimentation of the red cells was hastened by placing the tubes in the refrigerator so as to make the readings more precise.

After determining the dosage of antigen to be used in the final test, the latter is carried out in the following manner: To each of a series of tubes containing the inactivated serum in descending doses is added 0.05 cc. of complement in 1.5 cc. of saline solution followed by the proper dose of antigen. After incubation for a period of $\frac{1}{2}$ to 1 hour, the sensitized corpuscle suspension is added to each tube in the dosage already given, the mixture reincubated for 1 or 2 hours, and the readings are made as already indicated.

The customary controls for the serum, antigen, and hemolytic system were employed.

Non-specific fixations of the complement by rabbit sera, so frequently observed with bacterial antigens, must, of course, be kept in mind. Such a possibility, however, was ruled out by careful preliminary tests of the serum before immunization with numerous antigens, a procedure recommended by Kolmer and Trist (9). For various reasons it was not possible to select only those rabbits whose sera showed at the outset negative reactions. But we consider that this is unnecessary in serial studies, inasmuch as non-specific fixation does not interfere with specific deviations of the complement due to the presence of immune bodies.

Tests for Antistaphylolysin.—The staphylolysin used in the tests was prepared from a recently isolated strain of *Staphylococcus aureus* grown on a medium having an ionization equal to the value $P_H^{+} = 7.7$. Two units of this hemotoxin suspended in isotonic salt solution were added to a series of test tubes containing the inactivated serum in descending doses. The total volume was now made up to 2 cc., and the mixtures were incubated for 15 minutes in a water bath at 37° C. To each tube was then added 0.05 cc. of a suspension of red blood corpuscles prepared by washing the cells and adding an amount of saline solution equal to the original blood volume. The mixtures were now incubated for 1 hour. The readings were made at the end of 2 hours and again after 12 hours, the figures in the tables indicating the dilutions of serum in which the lytic activity of the staphylohemotoxin was completely inhibited.

Quantitation of Serum Proteins.—Those who have heretofore studied the problem of the relationship of the blood proteins to immunity have in the main obtained their data by precipitation of the globulins and the subsequent Kjeldahl determinations of the nitrogen contained in the precipitate and in the coagu-

lated proteins of the whole serum. Other workers have resorted to the less accurate method of weighing the precipitates. Neither of these procedures is applicable to a systematic study requiring frequent observations upon small animals because of the need of large quantities of blood and the time-consuming character of these procedures.

All the determinations of the albumin, globulin, and non-protein constituents in the blood of the animals experimented upon by us were made by the micro-refractometric method of Robertson (10). As the author has shown in numerous publications, the results obtained by this method are in accord with those obtained by the older methods, and the procedure possesses the important advantages of being less laborious and of being applicable to small quantities of serum.

In brief the method is as follows: Blood is collected in centrifuge tubes, allowed to clot, and centrifugalized to obtain a clear serum. The blood should be obtained before a feeding, since lipemic sera are read with more difficulty. Furthermore, the serum should not be allowed to remain in contact with the clot for any length of time, nor should bacterial contamination be permitted, especially if it is desired to keep the serum for 24 or 48 hours before analyzing it.

By actual experiment we became assured that serum, and more particularly immune serum, may dissolve out substances from the clot which may considerably alter its refractive index. It has been found, for instance, that after 48 hours a sterile immune serum⁴ kept in contact with the clot at low temperatures already showed a reduction in the protein quotient. On the other hand, the clear serum, immediately separated from the clot and kept under similar conditions, showed no marked changes after a period of 72 hours. A little over 1.5 cc. of serum is sufficient for the determination of the four fractions.

The tests are carried out in glass tubes having an inside diameter of about 5 mm. and walls about 1 mm. thick. These are sealed at one end.

For the determination of the albumin and globulin, 0.5 cc. of a saturated solution of ammonium sulphate is introduced with the aid of a graduated pipette into one of the tubes, about 10 cm. in length. With the same pipette, which has been cleaned by washing with water, alcohol, and ether, and dried by passing through it a stream of cold air, is added the same amount of clear serum. For purposes of mixing, a piece of silver wire is dropped into the tube, a stopper consisting of a piece of sealed glass tubing inserted into a piece of rubber tubing is affixed and the mixture of serum and sulphate is now shaken thoroughly. The precipitate of globulin is sedimented by centrifugalization, the clear fluid is diluted with a graduated pipette to one-half and its refractive index determined.

⁴ For details concerning the various steps in the method, and for a discussion of the reasons for them and of the manner of calculating the results, reference should be made to Robertson (10).

⁴ This immune serum exhibited a high antibody content. On May 8 the serum agglutinated in a dilution of 1:4,000, and fixed the complement in a dilution of 0.001 cc. of serum.

This reading corrected for the ammonium sulphate gives the total albumin plus the non-protein.

The non-protein value is determined by mixing in a similar glass tube 0.5 or 1 cc. of the clear serum with an equal volume of 0.04 N acetic acid solution. A short piece of silver wire is now dropped into the tube, the upper end is sealed off in the flame, the mixture shaken, and coagulated by placing the tubes in a beaker of water heated to boiling for several minutes. This precipitate also is sedimented by centrifugalizing and the refractive index of the clear supernatant fluid is determined.

Lastly the refractive index of the whole serum is determined. From this reading the refractive index of the total globulin is obtained by subtracting the refractive reading of the albumin from that of the whole serum after deducting the value of the non-proteins.

The readings were made with a Pulfrich refractometer, and the calculations of the percentages of the various constituents were carried out in the manner presented in detail by Robertson. The results are expressed not only in percentage but also in the per cent of total protein. For purposes of graphic presentation, it was thought well to express the ratio of albumin to globulin in the form of a quotient. This was obtained by dividing the percentage of albumin by that of globulin. Thus a fall in the quotient would indicate a rise in the blood globulins, and *vice versa*.

From a large series of determinations, numbering several hundred, we have become convinced of the accuracy of this method, provided the sources of error are understood and proper care is exercised in the manipulations. The method is especially recommended on account of the rapidity with which the determinations can be made and the small quantities of serum required.

EXPERIMENTAL.

Observations on the serum proteins were made in normal, infected, immunized, and hyperimmunized animals. With the exception of some of the infected animals, a parallel study was made also of the degree of immunity present during different periods of the experiment. As a typical example of an acute infection, staphylococcus pyemia was chosen. Infections with the tubercle bacillus and with sporothrix were selected as types of chronic infections.

For purposes of immunization living and killed cultures of *Bacillus typhosus* and *Bacillus dysenteriae* (Shiga) and *Staphylococcus pyogenes aureus* were used. In addition to the classical method of immunization, a study was made also of the effect of massive inoculations in normal and immune animals upon the albumin-globulin ratio and

upon antibody formation. To these were added several observations upon the changes produced in the serum proteins by the inoculation of bacterial endotoxins and inflammatory irritants.

Serum Proteins of Normal Rabbits.

Observations on the serum proteins were made on a dozen normal rabbits kept under constant conditions of diet and activity. Notwithstanding the constancy of the conditions, it is apparent that individual animals may show considerable variations in the percentage of the serum proteins. Thus the total proteins may vary from 5 to 7 per cent. The albumin fraction may show fluctuations from 3.1 to 5.5 per cent, and the globulins from 0.8 to 2.7 per cent. But the averages of all the readings yield values which are in fair accord with those of other workers (11), especially in so far as the albumin-globulin ratio is concerned. Such fluctuations as have been observed in the protein quotient in normal animals are small in comparison with the marked diminution in the quotient which has been noted in the pathological conditions studied. Whereas the quotient in normal animals averaged 3.5, and in most instances did not fall below 1.5, infected and immunized animals have at one period or another shown a quotient below 1.0. Furthermore, it is essential in all these experiments to determine the percentages of the serum proteins existing in the normal animal before proceeding to determine the variations which may follow the establishment of a pathological condition.

Infection.

Experiment 1. Infection. Pyemia.—Two rabbits were inoculated intravenously with potato cultures of *Staphylococcus pyogenes aureus*. One of the animals (Rabbit 1) received on February 17 two loopfuls of a culture, and died 8 days later of a bilateral fibrinous pleurisy, and abscesses in the lungs and kidneys. The second animal (Rabbit 2) was inoculated on March 2 with only one loopful of a potato culture. This animal emaciated gradually, losing about 600 gm. in weight, and was killed 13 days later.

Autopsy.—Animal anemic and emaciated. Pleural and peritoneal cavities contain a considerable amount of fluid. Atrophy of mesenteric fat. Slight engorgement of liver and spleen. Abscesses and infarcts in both kidneys. Large abscess in muscles of right hind leg. Fibrinopurulent arthritis of right coxofemoral joint. Bone marrow gelatinous and deep red in color.

The serum proteins were studied at frequent intervals during the course of the infection, and the results obtained showed clearly the fluctuations which may occur in the blood proteins in a typical infection with *Staphylococcus aureus*. The progress of the infection in both animals studied was accompanied by some antibody response as evidenced in the formation of antistaphylolysins. These developed to an equal degree in both rabbits. In Rabbit 2, however, the changes in the percentages of the various constituents, and especially in the albumin-globulin ratio, were not marked. The striking result of this experiment was the precipitous drop in the protein quotient observed in Rabbit 1, which received a massive inoculation of staphylococci. Of interest, too, is the fact that this rise in the total globulins was associated with a parallel fall in the total albumin.

The loss in weight in both animals was considerable, but it is of interest that this loss occurred more rapidly in Rabbit 1 (3 days), whereas as in Rabbit 2 it was more gradual. The significance of this observation will become more apparent in connection with other experiments.

Experiment 2. Tuberculosis.—On February 17 a rabbit weighing 2,600 gm. was inoculated intravenously with 0.1 mg. of a 72 day culture of bovine tubercle bacillus grown on tuberculin agar. During the progress of the infection, frequent analyses of the serum proteins were made, and the albumin-globulin ratio was determined. These observations are presented in Table I.

The animal emaciated only moderately, and was killed about 2½ months later.

Autopsy.—Extensive pulmonary tuberculosis with cavity formation. Numerous cheesy foci and miliary tubercles. Tuberculosis of mediastinal lymph nodes. Tubercles in spleen and kidneys.

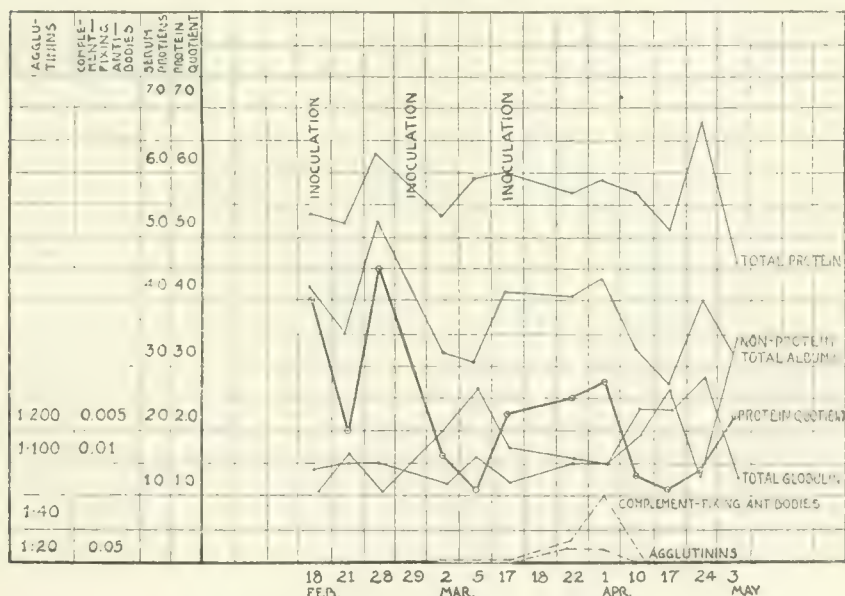
Experiment 3. Infection with Sporotrichum schenckii and beurmani.—A rabbit weighing 1,750 gm. was injected intraperitoneally with 2 cc. of a cream suspension of sporothrix (No. 3725, 1912) grown in 4 per cent glucose broth since October 25, 1915. The animal gained in weight and 10 days later 4 cc. of the same culture were injected. On the 30th day a third and final injection of 6 cc. was given. On March 17 three nodules about the size of large cherries were felt at the site of inoculation.

Frequent observations were made upon the agglutinins and complement-fixing antibodies as well as upon the serum proteins (Text-fig. 1). The animal was killed about 2½ months later.

Autopsy.—At the site of the injections there were many pea-sized nodules. These were present both in the subfascial layers and in the abdominal muscles.

TABLE I.
Experiment 2. Chronic Infection. Tuberculosis. Rabbit 3.

Date.	Weight.	Organisms inoculated	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		per cent	per cent	per cent	per cent	per cent	per cent		
Feb. 17..	2,600	0.1 mg. intra-venously.	6.6	5.3	1.3	80	20	1.2	4.0	Bovine strain grown on tuberculin agar, 72 days old.
" 21..	2,400	—	6.0	4.6	1.4	76	24	1.5	3.1	
" 25..	2,625	—	—	—	—	—	—	—	—	
" 28..	2,400	—	6.5	4.7	1.8	72	28	1.6	2.9	
Mar. 9..	2,700	—	6.0	4.2	1.8	70	30	1.4	2.3	
" 17..	2,400	—	6.8	4.6	2.2	68	32	1.4	2.1	
Apr. 1..	2,550	—	7.2	4.4	2.8	61	39	1.5	1.6	
" 10..	2,550	—	6.0	3.1	2.9	51	49	2.6	1.0	
" 17..	2,450	—	5.2	2.1	3.1	40	60	3.5	0.66	
" 24..	2,350	—	6.9	3.6	3.3	52	48	1.7	1.1	
May 3..	2,300	—	5.0	0.5	4.5	10	90	3.5	0.1	Animal killed. Blood for examination obtained from heart.



TEXT-FIG. 1. Infection with sporothrix.

Similar ones were found also in the omentum, between the loops of intestine and between the liver and diaphragm. Histologically, they were found to show the presence of typical sporotrichotic granulation tissue. From one of these nodules a positive culture was obtained.

Both the tubercular and mycotic infections were characterized by a long chronic course of several months associated with only slight wasting. Immediately following the inoculation, in the one instance with the tubercle bacillus and in the other with the sporothrix, each animal showed a slight rise in the total globulins as evidenced by a fall in the protein quotient. But it is of interest that the latter continued at a fairly constant level, and that the fluctuations observed usually followed the intraperitoneal injections. Thus it will be noticed the protein quotient did not show such a precipitous fall in the chronic as in the acute infections.

In the animal infected with sporothrix a slight grade of immunity developed, but neither the agglutinins nor the complement-fixing antibodies ever rose to a high level. Notwithstanding this lack of response, the serum proteins still showed striking fluctuations. The curve of total albumin for the most part paralleled closely the curve of total proteins. The globulins showed a tendency to rise when the albumin curve fell, but this was not the case for all periods of the experiment. Both of these features are well represented by the fluctuations of the protein quotient as shown graphically in the text-figure.

Immunity.

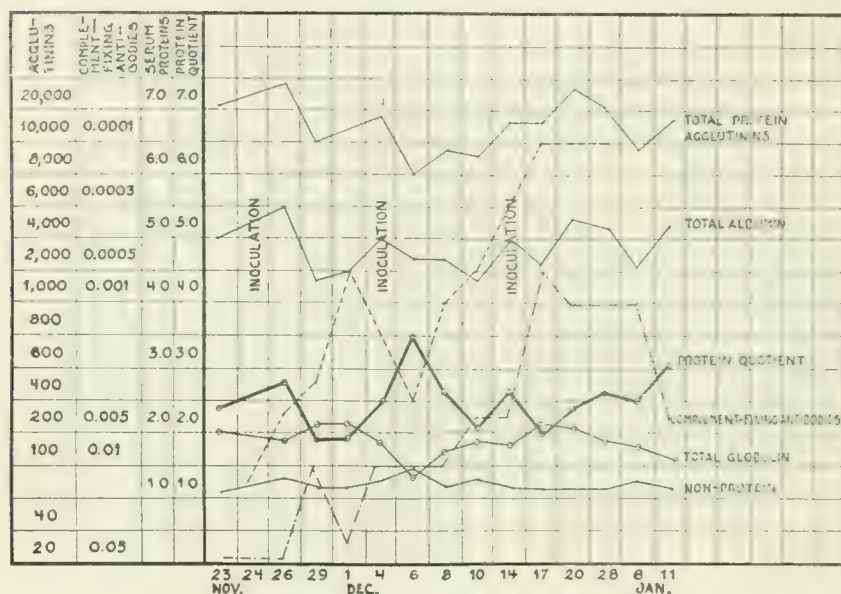
Experiments 1 and 2. Immunization with Bacillus typhosus.—Two healthy rabbits were inoculated with *B. typhosus*. One animal received inoculations of stock strain and the other Army vaccine. Serum samples were obtained before the injection for a study of the agglutinins, complement-fixing antibodies, and serum proteins. Similar observations were made at frequent intervals following the inoculations. The details of Experiment 2, in which Army vaccine was used, are recorded in Table II and Text-fig. 2.

In both of the experiments on typhoid immunization the inoculations were given in such dosage and at such intervals as to develop within the organism a maximum degree of immunity without causing any marked metabolic disorder. Both animals maintained their weight at a normal level throughout the period of immunization, and

TABLE II.
Experiment 2. Typhoid Immunization. Rabbit 4.

Date.	Organisms inoculated.	Antibodies.		Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
		Agglutinins.	Complement fixing antibodies.								
Nov. 23.	—	1:20	0.05	7.0	5.0	2.0	71	29	1.1	2.4	Army vaccine. Weight 3,800 gm.
" 24.	0.5 cc. (250,000,000) intravenously.	1:80	0.05	—	—	—	—	—	—	—	
" 26.	—	1:320	0.05	7.4	5.5	1.9	74	26	1.3	2.8	Weight 3,400 gm.
" 29.	—	1:500	<0.01	6.5	4.3	2.2	66	34	1.2	1.9	
Dec. 1.	—	1:2,000	0.03	6.7	4.5	2.2	67	33	1.2	2.0	
" 4.	—	1:800	0.01	6.9	5.0	1.9	72	28	1.3	2.5	
" 5.	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	Weight 3,800 gm.
" 6.	—	1:400	<0.01	6.0	4.7	1.3	78	22	1.4	3.5	
" 8.	—	1:1,000	<0.01	6.4	4.7	1.7	73	27	1.2	2.7	
" 10.	—	1:2,000	0.003	6.3	4.4	1.9	69	31	1.3	2.2	
" 14.	—	1:6,000	0.003	6.8	5.0	1.8	73	27	1.2	2.7	
" 15.	1.0 cc. (500,000,000) intravenously.	—	—	—	—	—	—	—	—	—	
" 17.	—	1:10,000	<0.0005	6.8	4.6	2.2	67	33	1.2	2.0	Weight 3,500 gm.
" 20.	—	1:10,000	0.001	7.4	5.3	2.1	71	29	1.2	2.4	
" 28.	—	1:10,000	0.001	7.1	5.2	1.9	73	27	1.1	2.7	
Jan. 6.	—	—	0.001	6.4	4.6	1.8	72	28	1.3	2.5	
" 11.	—	—	0.003	6.8	5.2	1.6	76	24	1.1	3.1	

the immunity developed after the third inoculation was of a high grade in each instance. In one of these animals (Rabbit 5), the agglutinins showed a tendency to fall at one period following an inter-current infection resulting from an abortion. Soon after this reduction in the agglutination titer, there occurred also a definite rise in the serum globulins. The association of the development of a pyemia with a high globulin content has been a frequent observation. Its significance will be discussed in subsequent paragraphs.



TEXT-FIG. 2. Typhoid immunization.

In neither animal was it possible to demonstrate any direct parallelism between the rise in the immune bodies and the fluctuations in the serum globulins. The latter showed a tendency to rise (fall in protein quotient) 24 to 48 hours following an inoculation, and a tendency to return to a normal level in the following several days.

Text-fig. 2 represents these fluctuations graphically for Experiment 2. It will be observed that after a period of about 3 weeks following the initial inoculation the value of the protein quotient showed no tendency to change materially, although the development of immune

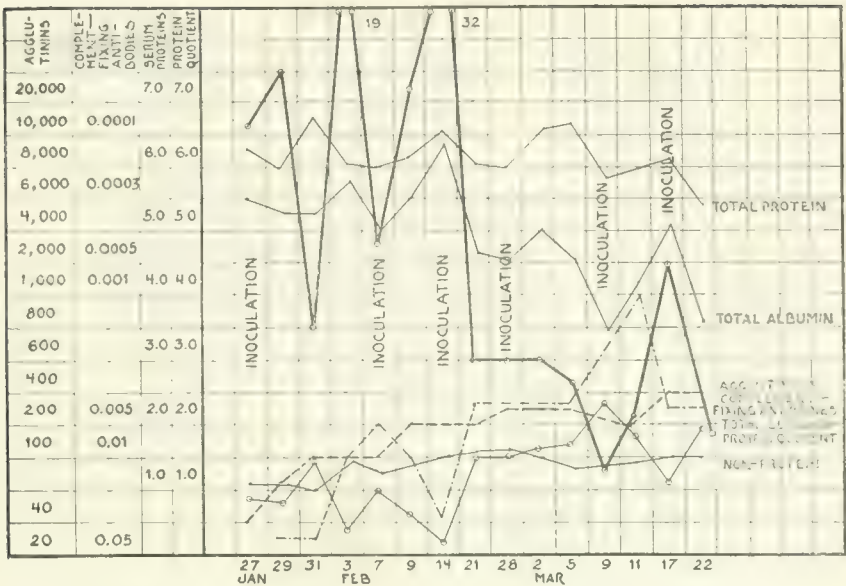
bodies had reached its highest point. The total proteins and total albumins showed parallel fluctuations but no definite tendency to rise during the process of immunization.

Experiment 3. Immunization with Dysentery Bacillus (Shiga).—A rabbit weighing 2,450 gm. was inoculated intravenously with increasing doses of living dysentery bacilli (Strain Do, Pasteur Institute, December, 1913) suspended in salt solution. The first inoculation was given on January 27 when 0.01 of a loop (20,000 organisms) was inoculated. The same dose was given 10 days later. 20 days after the first inoculation the animal received ten times this number of organisms. This was increased to 100 times the dose on the 30th day. On the 40th and 50th days, two and one-third and eight loopfuls, respectively, were inoculated. These injections were all well tolerated and were not followed by loss in weight. The degree of antibody response and the change in the serum proteins are recorded in detail in Table III and Text-fig. 3.

8 weeks after the beginning of the experiment, the animal died of exsanguination following prolonged bleeding from the ear artery.

The striking changes in the blood globulins brought about by the inoculation of living dysentery bacilli are well shown in the text-figure. It will be observed that following the first two inoculations both the albumin and globulin curves showed wide fluctuation, and that only after the third inoculation did the globulins show a gradual upward course and the albumin a gradual downward course. During two periods of the experiment (February 3 and 14) the albumin fraction rose to a high level. A similar observation was made upon an animal immunized with living staphylococci. Apart from the explanation that the injection of living organisms may give rise to a marked metabolic disorder, the reasons for such extreme variations in the curve are not clear, unless it is assumed that the active multiplication of bacteria may bear some relation to these fluctuations.

The agglutinins and complement-fixing antibodies rose gradually reaching their highest level during the 5th week. But as a careful analysis of the antibody and globulin curves will disclose, there is a marked fluctuation of the latter curve throughout its entire course. The most striking discrepancy was noted on March 17, when the concentration of antibodies had reached its maximum; whereas the globulin content was beginning to return to its initial level.



TEXT-FIG. 3. Immunization with living dysentery bacilli.

Experiments 4 and 5. Immunization with Living and Killed Staphylococci.—Two healthy animals were inoculated intravenously with *Staphylococcus pyogenes aureus*. One rabbit received 0.001 of a slant (2,000,000 organisms) at the beginning and two subsequent inoculations of 0.02 and 0.1 of a slant. This animal at autopsy showed an osteomyelitis of the sternum, an adhesive pericarditis, and thrombophlebitis of the deep femoral vein. The second animal was injected with cultures of staphylococci killed by heating at 60° C., for one or more hours. In all, four inoculations of 0.01, 0.2, 0.5 and 1 slant were given. The animal died on the 40th day of a septicemia following a hypopyon due to accidental injury.

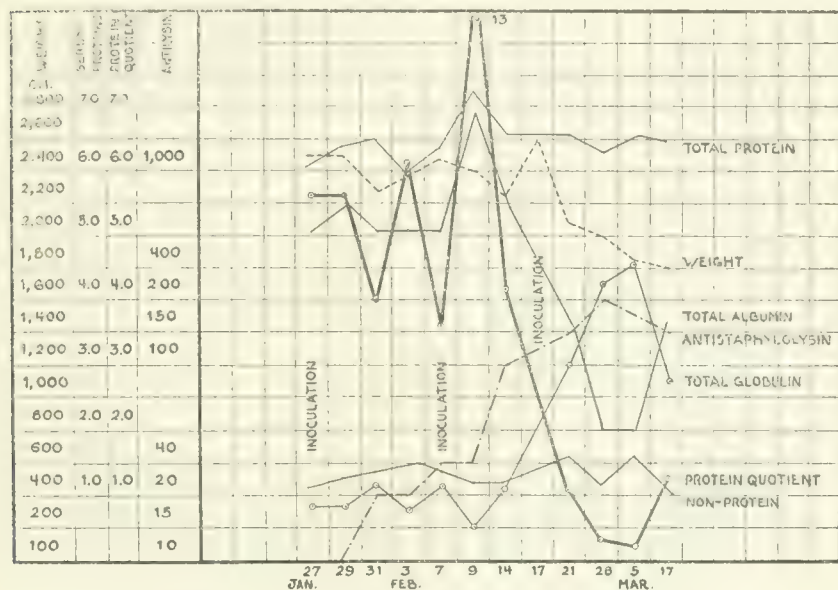
Some interesting differences have been observed between the effects produced by the intravenous inoculation of living and of killed staphylococci. The injection of living organisms is followed immediately by marked fluctuations in the protein quotient (Text-fig. 3), whereas following the injection of killed organisms the globulins first diminish and then the quotient shows a gradual downward course.

In Experiment 4 the injection of living organisms was followed on the 13th day by a marked rise in the albumin fraction. A similar

TABLE IV.
Experiment 5. Immunization with Killed Staphylococci. Rabbit 7.

Date.	Weight. gm.	Organisms inoculated.	Antibodies.		Total protein per cent	Total albumin. per cent	Total globulin. per cent	Albumin of total protein. per cent	Globulin of total protein. per cent	Non-protein constituents. per cent	Protein quo- tient.	Remarks.
			Agglu- tins	Comple- ment fixing bodies.								
Jan. 27.....	2,400	0.01 slant intrave- nously.	0	0	5.8	3.7	2.1	64	36	1.2	1.7	
" 29.....	2,400	—	0	0	5.7	4.2	1.5	73	27	1.5	2.7	
Feb. 3.....	2,350	—	0	0	5.4	4.1	1.3	76	24	1.5	3.1	
" 7.....	2,450	0.2 slant intrave- nously.	1:10	<0.2	—	—	—	—	—	—	—	Injury on left eye.
" 9.....	2,250	—	1:40	<0.2	6.6	5.7	0.9	86	14	1.4	6.1	
" 14.....	2,300	—	1:100	0.2	6.6	5.9	0.7	90	10	1.4	9.0	
" 21.....	2,600	—	1:60	>0.2	5.9	4.5	1.4	76	24	1.5	3.1	
" 22.....	2,850	0.5 slant intrave- nously.	—	—	—	—	—	—	—	—	—	
" 28.....	2,550	—	1:100	0.05	6.7	4.2	2.5	62	38	1.6	1.6	Suspension heated for 1 hr. at 60°C.
" 29.....	2,600	1 slant intrave- nously.	—	—	—	—	—	—	—	—	—	
Mar. 2.....	2,600	—	—	—	—	—	—	—	—	—	—	
" 6.....	2,300	—	1:200	0.01	7.8	2.4	5.4	31	69	1.6	0.45	

event occurred also in Rabbit 7 inoculated with killed organisms. But this animal had developed a suppurative condition of the left eye about the time that this sudden rise occurred. These two observations taken in conjunction with the one following the inoculation of living dysentery bacilli suggest the possibility that such extreme fluctuations may be explained by the active multiplication of living organisms within the animal.



TEXT-FIG. 4. Immunization with living staphylococci.

Both experiments illustrate still another point which will be considered more fully later; namely, that the increase in globulins is associated with a diminution in the albumin fraction. The total proteins exhibit a slight upward course in each animal, whereas the non-protein constituents show no significant variation.

A gradual rise in antistaphylolysin took place in Rabbit 8 inoculated with living organisms. For the most part, this rise appears to parallel the increase in globulins, but we feel that there is another consideration to be kept in mind in the interpretation of this result. The experimental evidence would seem to indicate that marked

alterations in weight, such as occurred in this animal, may be associated with a great increase in the globulins of the blood independently of a rise in immune bodies.

A Comparison of the Effect of the Inoculation of Living Typhoid Bacilli upon the Normal and Immune Animal.

The observations upon the fluctuations in the serum globulins and in the antibody response in normal animals immunized with living organisms suggested the problem of the possible effect of inoculating living organisms into the typhoid immune animal. That the immunized organism because of the sensitization of its fixed tissue cells may possess a more responsive defensive mechanism is now well known (12). This power of defense may become manifest by a rapid mobilization of antibodies and by a large increase in the number of circulating leukocytes. It was our purpose in the experiments of this series to ascertain whether this protective reaction was in any manner related to the changes in the blood proteins, and more especially whether any parallelism existed between the rise in leukocytes and the increase in the blood globulins.

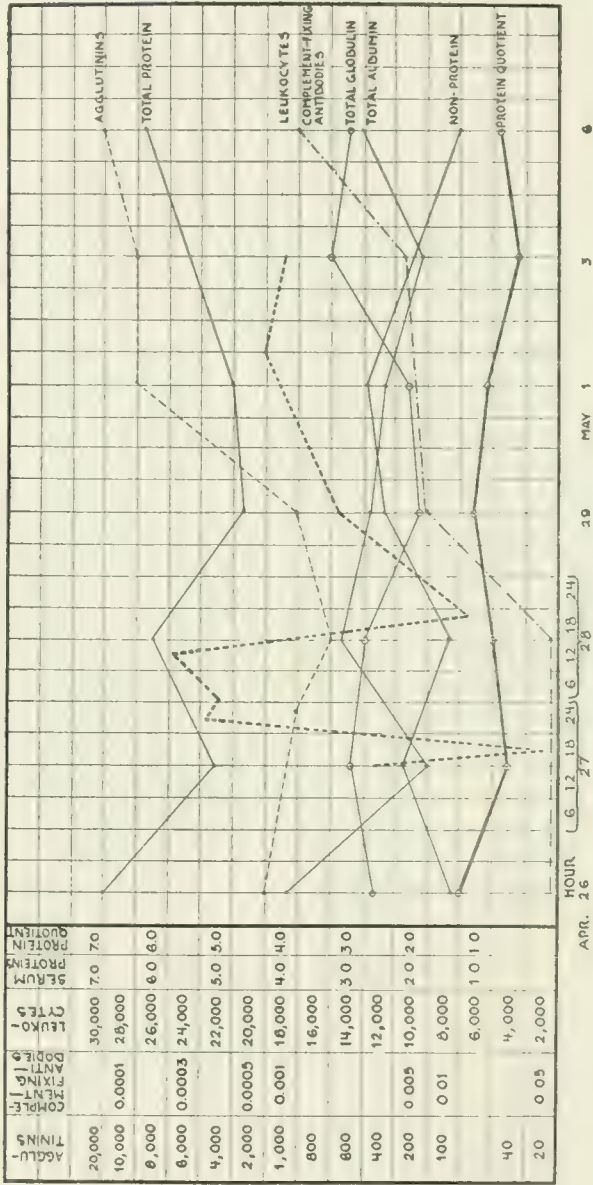
Experiments 1, 2, 3, 4, and 5.—Five animals already possessing a basic immunity against the typhoid bacillus were chosen for this study. In three of the animals (Rabbits 9, 10, and 4) a record was made at intervals of hourly periods of changes in the leukocytes, antibodies, and serum proteins. In one of the experiments (Rabbit 10) the observations were extended over a period of 12 days. The results of this experiment are representative of the others in this series. These are given in detail in Table V and Text-fig. 5.

Experiment 6.—A normal rabbit weighing 2,800 gm. was inoculated intravenously with 0.25 of a slant of living typhoid bacilli (Strain H 125) on May 8. The animal tolerated the injection well, and gave a good leukocytic and antibody response. Observations on the leukocytes, antibodies, and serum proteins were made at hourly periods during the first 48 hours, and then at intervals over a period of 16 days.

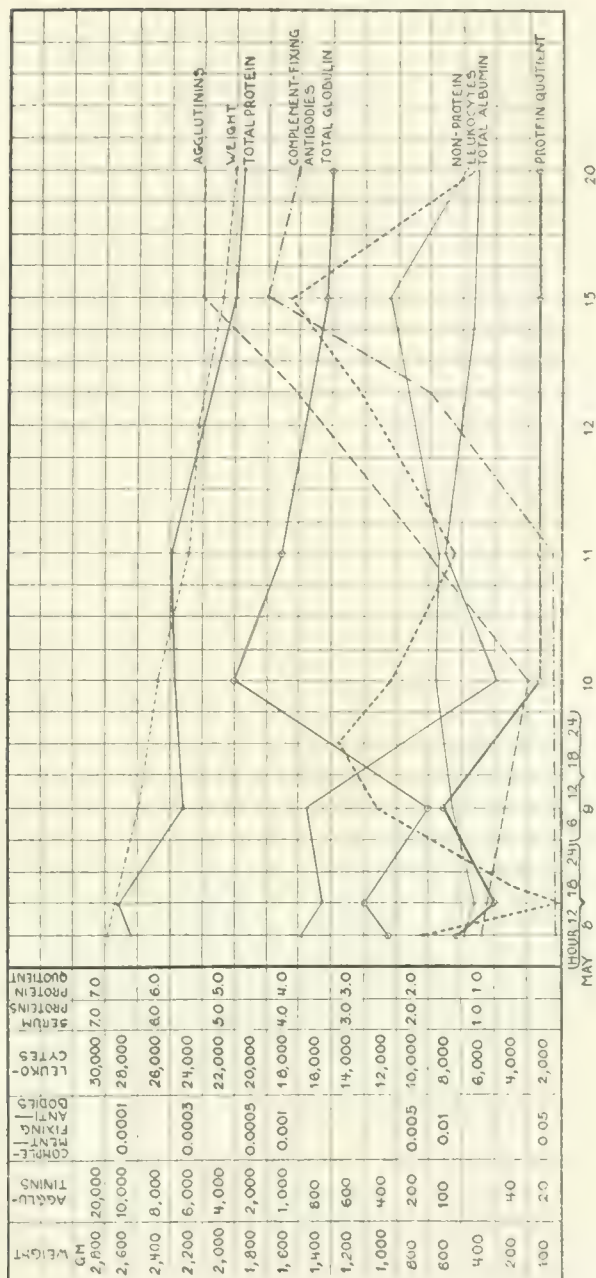
The observations given in the protocols, tables, and text-figures of this series support the general conclusions that the immune animal responds more quickly to the inoculation of living organisms with a leukocytosis, a rise in immune bodies, and an increase in the blood globulins; and that the changes noted in the blood proteins bear no relation to the hyperleukocytosis.

TABLE V.
Experiment 4. Rabbit 10.

Date.	Time.	Weight gm.	Antibodies.		Leukocyte count.	Total protein.		Total albumin.		Total globulin.		Albumin of total protein.		Globulin of total protein.		Non-protein constituents.		Protein quo- tient.	Remarks.
			Agglutinins.	Complement- fixing antibodies.		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent				
Apr. 26.....	—	—	1:2,000	0 05	—	7 0	4 1	2 9	60	40	1 6	1 5	—	—	—	—	—	Inoculation of 0.25 slant of 20 hour typhoid culture in- travenously.	
" 27.....	12 m.	3,475	—	—	12,300	—	—	—	—	—	—	—	—	—	—	—	—		
" 28	4 p.m.	—	—	—	3,770	—	—	—	—	—	—	—	—	—	—	—	—	<i>B. typhosus</i> in blood. " " " "	
	5.30 p.m.	—	1:1,000	0 1++	—	5 3	2 0	3 3	37	63	2 4	0 6	—	—	—	—	—		
	8.30 p.m.	—	—	—	23,000	—	—	—	—	—	—	—	—	—	—	—	—		
	9 a.m.	—	—	—	24,600	—	—	—	—	—	—	—	—	—	—	—	—		
	12.10 p.m.	—	1:800	0 05	18,600	6 2	3 2	3 0	51	49	1 7	1 0	—	—	—	—	—		
" 29	6 p.m.	3,350	—	—	7,400	—	—	—	—	—	—	—	—	—	—	—	—		
	—	—	1:1,000	0 005++	14,900	4 9	2 8	2 1	57	43	2 6	1 3	—	—	—	—	—		
May 1.....	—	3,750	1:10,000	0 003++	19,700	5 0	2 7	2 3	54	46	2 7	1 1	—	—	—	—	—		
" 3.....	—	—	1:10,000	0 003++	19,000	5 7	2 2	3 5	38	62	2 4	0 6	—	—	—	—	—		
" 6.....	—	—	1:20,000	0 001	—	6 3	3 0	3 3	47	53	1 5	0.88	—	—	—	—	—		



TEXT-FIG. 5. Injection of living typhoid bacilli. Immune animal.



TEXT-FIG. 6. Injection of living typhoid bacilli. Normal animal.

In the main the details of some of the experiments are given in Table V and Text-figs. 5 and 6, but a few points relating to the individual experiments deserve special mention. From a comparison of the results obtained in the experiments with Rabbits 11 and 12 it would appear that the rapidity of response bears some relation to the degree of the initial basic immunity. The first animal showed a fall in the agglutination titer immediately after the injection, and in this animal the rise in globulins occurred only after 72 hours. In Rabbit 12, however, the antibodies rose steadily, and in this instance a rise in the concentration of the serum globulins took place more rapidly within a period of 24 hours.

Experiments 3 and 4 are more complete since in them the observations were extended over a longer period. That the degree of hyperleukocytosis is dependent in part at least upon the number of organisms inoculated is clear from a comparison of the results obtained in Rabbits 9 and 10. In the former where the more marked reaction occurred, one-half of a standard agar slant had been inoculated; whereas, the latter received only one-quarter of a slant. The highest leukocytic reactions were observed in Rabbit 9 (44,000) and in Rabbit 4 (58,000). In this respect our observations coincide with those of McWilliams (12). The usual leukopenia which immediately follows the intravenous injection occurred in both the normal and immune animal as is graphically shown in Text-figs. 5 and 6.

By referring to these charts it will be noted that in neither animal was there any parallelism between the leukocytic response, the rise in the immune properties of the serum, and the increase in globulins. Whereas the curve showed periods in which a rise in globulins occurred simultaneously with a leukocytosis, the latter remained low even during the periods of leukopenia. Nor was any direct correspondence demonstrable between the rise in immune bodies and the increase in the concentration of the serum globulins. Both in the normal and the immune animal the latter took place long before any appreciable rise in the agglutinins and complement-fixing antibodies had occurred. In Rabbit 10, for instance, a fall in the protein quotient (globulin rise) was demonstrable within 24 hours after the inoculation at a time when the antibody content was at its lowest level; and similarly in Rabbit 13, the normal control, the serum globulins rose mark-

edly within 48 hours, while the antibodies rose to their maximum height only after 4 days. In both instances the globulin content remained high throughout the period of immunization while the antibody curve continued to rise independently of the globulins.

The advantage of such frequent determinations made at different periods during the process of immunization as compared with isolated observations made at random intervals is well illustrated in these experiments. If, for instance, a determination of the globulin fraction should show an increase at a time when the immune bodies had reached a high level, the conclusion would naturally follow that fluctuations in the two parallel one another; whereas, as we have pointed out, more frequent observations demonstrated that such parallelism was not of constant occurrence.

The tabulated results emphasize still another point of importance. To be of absolute value the albumin-globulin ratio must be expressed in terms of their quotient. This takes into consideration also fluctuations which have been found to take place in the total proteins during the course of an infection and during the process of immunization.

The Effect of the Injection of Bacterial Toxin upon the Serum Proteins.

The experimental data already presented afford evidence that both living and killed cultures of various bacteria when inoculated into an animal give rise to marked changes in the serum proteins, and more especially to an upset of the normal albumin-globulin ratio. This phenomenon may well be attributed to the changed condition of the animal's metabolism resulting from the multiplication of bacteria within its body, to the liberation of toxic products from the disintegration of the bacterial bodies, or to both causes. The part which the autolyzed bacterial bodies themselves may play in bringing about the results observed is well shown in the following experiment.

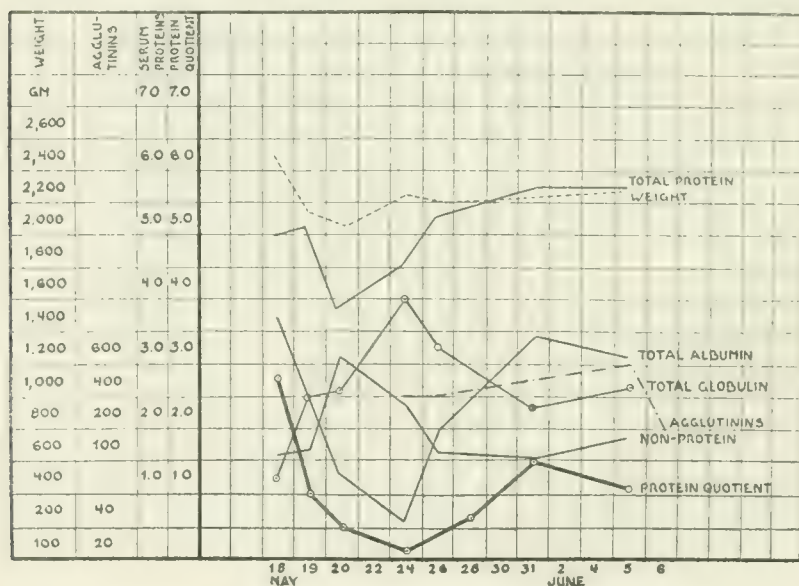
The Action of Bacterial Endotoxin.—A rabbit weighing 2,500 gm. was injected intravenously on May 18 with 1 cc. of the toxin of fowl typhoid. This toxin was prepared by growing the organisms on Martin's broth for 14 days, after which the culture was centrifugalized and filtered through a Berkefeld filter.

Specimens of 5 cc. of blood were taken 24 hours after the injection and at varying intervals until June 6. The alterations in the serum proteins following the injection are given in Table VI and Text-fig. 7.

TABLE VI.

The Effect of the Injection of Bacterial Toxin (Fowl Typhoid). Rabbit 14.

Date.	Weight.	Amount of toxin injected.	Agglutinins.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non protein constituents.	Protein quotient.	Remarks
	gm.			per cent	per cent	per cent	per cent	per cent	per cent		
May 18...	2,500	1 cc. intravenously.	—	5.0	3.7	1.3	74	26	1.6	2.8	Strain 605 grown on Martin's broth for 14 days. Centrifugalized and filtered once through a Berkefeld filter.
" 19...	2,175	—	—	5.1	2.6	2.5	51	49	1.7	1.0	
" 20...	2,100	—	—	3.9	1.3	2.6	33	67	3.1	0.5	
" 24...	2,275	—	1:400	4.6	0.6	4.0	13	87	2.4	0.15	
" 26...	2,225	—	1:400	5.3	2.0	3.3	37	63	1.7	0.6	
" 31...	—	—	—	5.7	3.4	2.3	60	40	1.6	1.5	
June 5...	2,300	—	1:600	5.7	3.1	2.6	54	46	1.9	1.1	
" 6...	—	—	1:200	—	—	—	—	—	—	—	



TEXT-FIG. 7. The effect of the injection of bacterial toxin.

The dosage of endotoxin in this experiment was apparently well chosen, for although the animal lost moderately in weight, the amount of toxin was not sufficient to prevent a gradual return of the serum proteins to a more or less normal state. The most striking effect of the inoculation, shown graphically in Text-fig. 7, was the gradual increase in the serum globulins at the expense of the albumin fraction, and a reduction in the percentage of the total proteins. This rise in globulins was already appreciable 72 hours following the injection, and 6 days later the albumin-globulin ratio still showed an inversion of the normal formula. On the 8th day the total per cent of proteins had returned to normal, and continued to rise somewhat above the normal level during the subsequent 11 days. At this time the protein quotient, however, still remained low, although the albumin-globulin ratio was beginning to revert to its normal state.

The tendency in this instance for an alteration in the blood protein fractions to readjustment is of interest. In this respect this observation is unique, for in the majority of the experiments an upset of the normal ratio continued with some fluctuations for a long period of time due to subsequent reinoculations. The readjustment of conditions in this animal may be attributed to the absence of bacterial invasion to perpetuate the process. It may be assumed that after its initial effect upon the animal, the toxin was spent, as may be inferred from the appearance of antibodies in the blood, and that the organism was then able to readjust itself.

A Comparison of the Effect of the Intraperitoneal Injection of Bacterial and of Inflammatory Irritants.

In some experiments which will be recorded at a later date, we observed that the intraperitoneal injection of red blood corpuscles gives rise to an alteration in the albumin-globulin ratio which is both rapid in its occurrence and marked in its degree. In fact such injections usually resulted in a complete inversion of the ratio within a period of 24 hours. Because we were dealing here with non-bacterial protein and with a different route of injection, it seemed worth while to ascertain whether it was the nature of the inoculated material or the route of the inoculation which was responsible for

the changes observed. With this purpose in view, the following two experiments were carried out.

Experiment 1.—On May 22 a rabbit weighing 2,525 gm. was injected intraperitoneally with 0.2 of a slant of a killed culture of staphylococci. The organisms were killed by heating for an hour at 60° C., and 1 cc. of the suspension was used. An analysis of the serum proteins was made at stated intervals following the injection. These are recorded in Table VII.

TABLE VII.

The Effect of the Injection of Killed Staphylococci Intraperitoneally. Rabbit 15.

Date.	Weight.	Organisms inoculated.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
			per cent	per cent	per cent	per cent	per cent	per cent		
May 19.	2,500	—	5.8	3.2	2.6	60	40	1.7	1.5	
" 22.	2,525	0.2 slant intraperitoneally.	—	—	—	—	—	—	—	24 hour culture killed by heating for 1 hr. at 60°C. suspended in 1 cc. of saline solution.
" 23.	2,500	—	4.7	1.3	3.4	28	72	2.9	0.4	
" 24.	2,600	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 26.	2,600	—	4.9	0.8	4.1	16	84	1.8	0.2	
" 31.	—	—	5.7	2.0	3.7	35	65	1.6	0.5	
June 6.	—	—	5.8	1.8	4.0	31	69	1.3	0.45	

Experiment 2.—2 cc. of an aleuronat suspension in saline solution were injected intraperitoneally into a rabbit weighing 2,150 gm. The suspension of aleuronat was so made that it corresponded in density to that of the killed staphylococci used in the first experiment. Following the injection, observations were made upon the serum proteins. These are recorded in Table VIII. The animal lost gradually in weight, and died 7 days after the injection.

Autopsy.—Small masses of unabsorbed aleuronat were found adherent to the peritoneum. There was a definite intestinal paralysis with coprostasis, chiefly in the large bowel. There was considerable injection of the peritoneum and an enteritis of the small bowel.

TABLE VIII.

The Effect of the Injection of Aleuronat Intraperitoneally. Rabbit 16.

Date.	Weight.	Amount of aleuro- nat injected.	Total protein.	Total albumin.	Total globulin.	Albumin of total protein.	Globulin of total protein.	Non-protein constituents.	Protein quotient.	Remarks.
	gm.		per cent	per cent	per cent	per cent	per cent	per cent		
May 19..	2,075	—	5.1	3.1	2.0	60	40	1.6	1.5	
" 22..	2,150	2cc.	—	—	—	—	—	—	—	Aleuronat (Merck) sus- pension made in saline solution and of about same density as that of staphylococci (Table VII).
" 23..	2,025	—	4.7	1.8	2.9	38	62	2.8	0.6	
" 24..	2,025	—	4.2	1.7	2.5	40	60	3.1	0.66	
" 26..	1,900	—	4.7	1.5	3.2	32	68	2.6	0.5	
" 29..	1,450	—	—	—	—	—	—	—	—	Death.

Both experiments would seem to support the view that the route of injection rather than the nature of the substance injected is responsible for the rapid inversion in the albumin-globulin ratio. 24 hours following the intraperitoneal injection of killed staphylococci the quotient fell from 1.5 to 0.4, indicating an increase in globulins to more than three times the initial value. This upset in the ratio continued with slight fluctuations for a period of about 2 weeks. The animal injected with aleuronat showed a change similar in every respect.

The retardation in response noted after intravenous inoculations must therefore be attributed to the protective properties of the blood which enable it to delay the action of the bacteria or toxin upon the body tissues.

Another point brought out by this experiment deserves emphasis; namely, that agents other than bacteria or their toxins may cause an upset in the serum proteins. The manner in which an inflammatory irritant and leukotactic substance like aleuronat may produce this result offers some difficulty of explanation. It is not unlikely, however, that the rapid absorption of toxic protein products result-

ing from the disintegration of leukocytes and fixed tissue cells produces a profound metabolic disturbance of which the heaping up of blood globulins is one of the resultant phenomena. A further consideration of the factors which may give rise to this result will be presented later.

DISCUSSION.

The experimental evidence presented does not support the views held by a number of workers concerning the relationship of the blood globulins to the resistance developed in bacterial infection and immunity. From a large number of observations, continued over a long period of time, we have become convinced that other causes are responsible for the rise in globulins observed in these conditions.

Our observations have shown with considerable certainty that a heaping up of globulins in the blood during the development of an infection is more apt to occur in those instances where the infection has been overwhelming and associated with extensive suppuration and wasting. We have found, in fact, that animals which succumb to such an acute process have usually developed only a moderate resistance as far as the development of immune bodies is concerned. On the other hand, a mild chronic infection may continue over a long period of time, and may register only slight changes in the blood globulins until the animal begins to emaciate and to lose in weight. This point has been discussed in connection with the tubercular and the mycotic infections.

The main points of interest have come from a study of the serum globulins during the process of immunization. Contrary to the results of a number of workers (13), our experiments have shown that immunization with bacteria causes a rise in globulins only when the animals react severely to the inoculation. Immunization carried out carefully and with a well controlled dosage is not usually accompanied by an increase in the serum globulins, although the immune bodies may attain a high concentration. The inoculation of massive doses, however, either into a normal animal or into an animal already possessing a basic immunity results in most instances in a marked rise in the globulins. This may occur, indeed, before the animal has responded by the production of antibodies. It would seem,

therefore, that no direct parallelism exists between the two phenomena. In fact we have come to regard the heaping up of serum globulins supervening during the process of immunization as an index of a metabolic disorder unfavorable to the attainment of the best immunologic results. And it is not unlikely that observations on the blood globulins may serve as an important practical guide to careful immunization.

Any attempt to explain the cause of the rise in globulins observed in infection and immunity is difficult. At best all such explanations must be of a hypothetical character until we have learned more concerning the origin of the various protein fractions, their function,⁵ and their chemical nature. One important conclusion may be derived from the experimental evidence presented; namely, that the increase in blood globulins is usually accompanied by a marked metabolic disorder. This observation has been made also by other workers (14). Clinically, the metabolic disturbance is manifested by a febrile reaction, intoxication, and rapid emaciation. The extensive destruction of body protein which is going on is further evidenced by an increase in the nitrogen elimination (15). That such marked proteolytic activity may be initiated by the intravenous injection of bacteria, bacterial toxins, and protein split-products has been clearly shown by Jobling and his coworkers (16). They attribute this active proteolysis to a more or less marked mobilization of ferments, both protease and lipase. Apart from the consideration of the cause of this disturbance in metabolism, it seems reasonable to assume that it must register a change in the proteins of the blood.

More difficult to explain, however, are the facts that an inversion of the albumin-globulin ratio can be so readily produced, and that the change markedly affects the globulins. The possible explanations which may be offered for these phenomena have some basis in experiment. Moll (17) has shown, for instance, that under optimum conditions of reaction and temperature crystalline albumin can be converted *in vitro* into a substance whose chemical and physical

⁵ Friedemann (*Z. Hyg. u. Infektionskrankh.*, 1910, lxvii, 279) thinks that the globulins and albumins of normal serum are in antagonism, the albumins preventing certain reactions, such as complement fixation, in which the former become active as soon as the albumins are removed or diminished.

properties correspond in every particular to a globulin. On the basis of this observation one would have to assume that the more rapid conversion of albumin into globulin within the body is only a part of the accelerated metabolism which takes place in infected and immunized animals.

This is only one of many hypotheses which might be advanced to explain the difficult questions which the recorded observations offer for consideration. But at the present time it is better to adhere to those views which have an experimental basis until further additions to our knowledge make them untenable.

SUMMARY.

The progress of an infection is usually associated with marked changes in the serum proteins. There may be an increase in the percentage of the total protein during some stage of the infection, and there is usually a change in the albumin-globulin ratio with an increase in the total globulins. This rise may antedate the development of any resistance by a considerable period of time.

The non-protein constituents of the blood show fluctuations with a tendency to rise as the infection progresses.

The process of immunization is in almost all instances associated with a definite increase in the globulins of the blood, and in some cases with a complete inversion of the normal albumin-globulin ratio. This may be produced both by living and dead organisms and by bacterial endotoxins. Massive doses usually result in an upset which shows no tendency to right itself during the period of observation.

A rise in the globulins has been shown to occur long before the animal develops immune bodies in any appreciable concentration; and where the globulin curve and antibody curve appear to parallel one another, it can be shown by a careful analysis of both curves that there is a definite lack of correspondence at various periods of the experiment.

Animals possessing a basic immunity show a more rapid rise in the globulin curve following inoculation.

There is no parallelism between the leukocytic reaction and the

globulin reaction. During periods of leukopenia the globulins may be as high as during the period of a leukocytosis.

Bacterial endotoxins produce as striking an increase in the serum globulins as do living and killed bacteria. This would seem to indicate that a bacterial invasion of the organism is not absolutely essential for the globulin changes, and that the toxogenic factor in infection and immunity must play a part in the production of the changes noted.

Inflammatory irritants injected intraperitoneally also result in a globulin increase. In this case the changes produced may best be explained by the toxogenic effect produced by the protein split products resulting from the inflammatory condition.

Intraperitoneal injections of killed bacteria give rise to a more rapid increase in the serum globulins. The rapidity of the response following intraperitoneal as compared with intravenous injections doubtless stands in intimate relationship to the neutralizing power possessed by the blood serum and perhaps to the more extensive surface of absorption following injection by the intraperitoneal route.

BIBLIOGRAPHY.

1. Brodie, T. G., A Preliminary Report of Some Experiments upon the Chemistry of the Diphtheria Antitoxin, *J. Path. and Bacteriol.*, 1896-97, iv, 460.
2. Seng, W., Ueber die qualitativen und quantitativen Verhältnisse der Eiweisskörper im Diphtherieheilserum, *Z. Hyg. u. Infectious-krankh.*, 1899, xxxi, 513.
3. Hiss, P. H., Jr., and Atkinson, J. P., Serum-Globulin and Diphtheria Antitoxin. A Comparative Study of the Amount of Globulin in Normal and Antitoxic Sera, and the Relation of the Globulins to the Antitoxic Bodies, *J. Exp. Med.*, 1900-01, v, 47.
4. Ledingham, J. C. G., On the Relation of the Antitoxin to the Globulin-Content of the Blood Serum during Diphtheria Immunisation, *J. Hyg.*, 1907, vii, 65. Gibson, R. B., and Banzhaf, E. J., The Quantitative Changes in the Proteins in the Blood Plasma of Horses in the Course of Immunization, *J. Exp. Med.*, 1910, xii, 411.
5. Pfeiffer, R., and Proskauer, B., Beiträge zur Kenntniss der spezifisch wirksamen Körper im Blutserum von choleraimmunem Tieren, *Centr. Bakteriolog., Ite Abt.*, 1896, xix, 191.
6. Pick, E. P., Zur Kenntniss der Immunkörper, *Beitr. chem. Phys. u. Path.*, 1902, i, 351, 393, 445.

7. Rodhain, J., Beitrag zur Kenntniss der wirksamen Substanzen des Antistreptokokkenserums, *Beitr. chem. Phys. u. Path.*, 1902-03, iii, 451.
8. Moll, L., Über Blutveränderungen nach Eiweissinjektionen, *Beitr. chem. Phys. u. Path.*, 1904, iv, 578.
9. Kolmer, J. A., and Trist, M. E., Studies in Non-Specific Complement Fixation. I. Non-Specific Complement Fixation by Normal Rabbit Serum, *J. Infect. Dis.*, 1916, xviii, 20.
10. Robertson, T. B., A Micro-Refractometric Method of Determining the Percentages of Globulin and Albumin in Very Small Quantities of Blood Serum, *J. Biol. Chem.*, 1915, xxii, 233.
11. Robertson, Studies in the Blood Relationship of Animals as Displayed in the Composition of the Serum Proteins. I. A Comparison of the Sera of the Horse, Rabbit, Rat, and Ox with Respect to Their Content of Various Proteins in the Normal and in the Fasting Condition, *J. Biol. Chem.*, 1912-13, xiii, 325. Wells, C. E., The Influence of Age and Diet on the Relative Proportions of Serum Proteins in Rabbits, *ibid.*, 1913, xv, 37.
12. Cole, R. I., Experimenteller Beitrag zur Typhusimmunität, *Z. Hyg. u. Infektionskrankh.*, 1904, xlv, 371. McWilliams, H. I., Is the Hyperleucocytosis Following the Injection of Typhoid Bacilli into Immunized Rabbits Specific, *J. Immunol.*, 1916, i, 159.
13. Langstein, L., and Mayer, M., Über das Verhalten der Eiweisskörper des Blutplasmas bei experimentellen Infektionen, *Beitr. chem. Phys. u. Path.*, 1904, v, 69.
14. Cervello, C., Einfluss der Antipyretica auf die Albuminoide des Blutserums, *Arch. exp. Path. u. Pharm.*, 1910, lxii, 357. Breinl, F., Beitrag zur Kenntnis der Serumeiweisskörper, *ibid.*, 1911, lxv, 309. Glaessner, K., Ueber das Verhalten des Blutglobulins beim Immunisierungsvorgange, *Z. exp. Path. u. Therap.*, 1906, ii, 154.
15. Friedemann, U., and Isaak, S., Ueber Eiweissimmunität und Eiweissstoffwechsel, *Z. exp. Path. u. Therap.*, 1905, i, 513.
16. Jobling, J. W., and Petersen, W., Bacteriotherapy in Typhoid Fever, *J. Am. Med. Assn.*, 1915, lxv, 515.
17. Moll, L., Über künstliche Umwandlung von Albumin in Globulin, *Beitr. chem. Phys. u. Path.*, 1904, iv, 563; Kürzere Mitteilungen. 5. Zur künstlichen Umwandlung von Albumin in Globulin, *ibid.*, 1906, vii, 311.

A STUDY OF THE BLOOD IN HEMOPHILIA*

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INTRODUCTION

During the past few years the etiology of the hemorrhagic diseases has been the subject of considerable study. The progress made in this direction has been due largely to an increase in our knowledge of the physiology of blood coagulation and to the development of new methods of study. The literature of this period, indeed, has even given promise of an etiologic classification of hemorrhagic disease, but to the present time this goal has only been partly attained.

It has been possible, for instance, to demonstrate that certain of the hemorrhagic conditions are associated with a deficiency in one or another factor of coagulation: there are on record instances of hemorrhagic disease associated with abnormalities in the prothrombin and antithrombin content of the blood.¹ There is also experimental evidence of the existence of certain types of hepatic disease with some tendency to abnormal bleeding due to a deficiency in fibrinogen.² But on the other hand there are a number of clinical conditions characterized by a tendency to bleed, which at the present time cannot be rigidly classified on the basis of a defect in the fibrin factors.

Certain forms of purpura, for instance, are attributed by most workers to a deficiency in the number of blood platelets. Studies on the factors of coagulation in this disease have for the most part yielded negative results.³ But repeated examinations of the blood of two patients with chronic purpura made over a period of six months have

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1. Whipple, G. H.: Hemorrhagic Disease. Antithrombin and Prothrombin Factors, *THE ARCHIVES INT. MED.*, 1913, xii, 637. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, *THE ARCHIVES INT. MED.*, 1914, xiii, 76. Drinker, C. K., and Hurwitz, S. H.: The Factors of Coagulation in Primary Pernicious Anemia, *THE ARCHIVES INT. MED.*, 1915, xv, 733. Following the completion of the present paper, there appeared an extensive study by Minot, Denny and Davis (*THE ARCHIVES INT. MED.*, 1916, xvii, 101) on the prothrombin and antithrombin factors in a large number of varied clinical conditions. Their observation that some types of purpura may show a high antithrombin content coincides with our experience.

2. Whipple, G. H.: Hemorrhagic Disease—Septicemia, Melena Neonatorum and Hepatic Cirrhosis, *THE ARCHIVES INT. MED.*, 1912, ix, 365.

3. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, *THE ARCHIVES INT. MED.*, 1914, xiii, 76. See also last paragraph of Note 1.

convinced us that certain chronic types of the disease may at times show blood abnormalities in addition to a platelet deficiency. Thus, in the patients studied by us, fluctuations were observed at different times in the amount of circulating antithrombin: periods of antithrombin excess occurred in waves during the course of the observations; but it could not be demonstrated that the increase in the amount of antithrombin was associated in any definite way with an aggravation in the severity of the clinical symptoms. These few observations are only suggestive, and from them alone no generalizations are permitted concerning the part played by the factors of coagulation in this type of hemorrhagic disease. It is not unlikely, however, that further studies may disclose other cases with a similar defect.

On the other hand, recent work has shown that the cause of hemophilia may be ascribed with considerable certainty to an abnormality in one of the factors of coagulation, and that the hemophilic condition is characterized by certain reactions of the blood which distinguish it from other hemorrhagic diseases. It is the purpose of this paper to present confirmatory evidence in support of this view.

VIEWS CONCERNING THE CAUSE OF HEMOPHILIA

The nature of the explanations offered for the causation of hemophilia varies with the theory of coagulation adopted. If we accept the theory of Howell, there are five factors concerned in the clotting of blood—prothrombin, antithrombin, thromboplastin, fibrinogen and calcium. All of these elements, except thromboplastin, are present in circulating blood. Within the blood vessels prothrombin is held in combination with antithrombin and intravascular clotting is thereby prevented. When, however, blood is shed, the antithrombin is neutralized by the thromboplastic substance of the tissue juices. The liberated prothrombin is now activated by the calcium and the thrombin which results converts soluble fibrinogen into the insoluble fibrin or the clot.

According to the opposing view of Morawitz, only four possible factors need be considered. The essential feature of this theory centers about the activation of thrombin, which, it is contended, results from prothrombin by the combined activity of calcium and a substance designated as thrombokinase. The existence of antithrombin in the circulating blood is admitted by Morawitz, but it is not regarded by him as an essential part of the process.

The main distinguishing feature of the hemophilic condition is the greatly prolonged coagulation time of the blood when it is removed from the vessels with the precautions necessary to prevent its contamination with tissue juices. All recent workers are agreed that this defect in coagulation is a constant pathological sign of the disease; but different explanations have been given by different workers to account

for the delayed coagulability. Hemophilia has been ascribed, indeed, to a deficiency in one or another of the various elements which take part in the clotting of normal blood. Without entering into a discussion of the experimental evidence given by each observer, it may be well to review briefly the essential fact of the various views presented.

Wright⁴ has attributed the hemophilic condition to a deficiency of calcium. Weil⁵ has given some experimental evidence for the view that the delayed coagulability of hemophilic blood may be caused by the presence of an excess of some coagulation inhibiting body. Sahli⁶ and Morawitz and Lossen,⁷ adopting the theory of coagulation proposed by Morawitz, have upheld the view that the essential defect in hemophilic blood may be ascribed to insufficient or defective formation of thrombokinase. This view is shared for the most part by Nolf,⁸ who believes hemophilic blood to be deficient in the substance designated by him as thrombozym (thrombokinase, thromboplastic substance). Aldis,⁹ however, has given experimental proof for the view that the hemophilic condition is due to a change in the properties of the circulating prothrombin, and Howell³ has concluded that not the property but the actual amount of prothrombin is altered in hemophilic blood.

During the past year we have had an opportunity to make complete blood studies in five cases of hemophilia. These patients all exhibited the hemophilic condition in marked degree, although it was not possible to obtain a definite history of hemophilia in the ascendants. A study of the blood, however, showed in each instance the characteristic delay in the coagulation time and a striking deficiency in prothrombin. From the tabulated results it will be seen that the other factors of coagulation were present in normal amounts. With one or two exceptions, the absolute amount of antithrombin present in hemophilic blood was seldom greater or less than that of normal blood; and the amount of fibrinogen was found to fluctuate little from the normal.

Our conclusions support the view that the deficiency in prothrombin is a constant characteristic of the hemophilic condition. On the basis of this observation, it is essential that a definition of hemophilia not only include the clinical characteristics of excessive and immoderate hemorrhage and its transmissibility as a sex-limited inheritance, but also in addition the especial reaction of hemophilic blood which distinguishes it from the blood of other hemorrhagic diseases also characterized by a tendency to bleed.

4. Wright, A. E.: *Brit. Med. Jour.*, 1893, ii, 223; *ibid.*, 1894, ii, 57.

5. Weil, Emile P.: *Presse méd.*, 1905, xiii, 673.

6. Sahli, H.: *Ztschr. f. klin. Med.*, 1905, lvi, 264; *Deutsch. Arch. f. klin. Med.*, 1910, xcix, 518.

7. Morawitz, P., and Lossen, J.: *Deutsch. Arch. f. klin. Med.*, 1908, xciv, 110.

8. Nolf, P.: *Ergebn. d. inn. Med.*, 1913, x, 275.

9. Addis, T.: *Jour. Path. and Bact.*, 1911, xv, 427.

METHODS OF STUDY

1. *Determination of the Coagulation Time.*—The coagulation time of the blood was determined according to the method employed by Morawitz and Bierich¹⁰ and by Howell. A specimen was obtained by venous puncture into a syringe coated with a thin layer of petrolatum-ether mixture. Of the sample obtained, 2 c.c. were expelled at once into a thoroughly cleaned test tube measuring about 13 to 14 mm. in internal diameter (the test tubes ordinarily used in bacteriologic laboratories serve well for this purpose). Tubes of the same diameter were used for all comparable determinations. The observations were made at a constant temperature (about 25 C.). Complete invertibility of the clot was taken as the end point of the reaction. In each instance comparison was made with a specimen of blood taken from a normal individual with the same precautions. By this method the average clotting time of normal blood obtained in twenty-one tests was twenty minutes with a minimum of eight minutes and a maximum of thirty-three minutes.

It may be well to emphasize the importance of painstaking technic in obtaining blood for studies on the factors of coagulation, since a careless procedure may lead to erroneous results. In studies on the fibrin factors, interest centers on these elements as they exist in the circulating blood rather than on their behavior when blood is shed. The latter information is obtained better by a determination of the bleeding time,¹¹ the duration of which depends on a number of additional factors: the potency of the tissue juices, the mechanical and chemical action of the blood platelets, and the elasticity of the skin.

When blood is obtained by skin puncture, admixture with tissue juices invariably results. These exert an influence the extent of which cannot be measured, for it is obviously difficult to make certain that control specimens will be exposed to the same wound surface, and receive the same amount of contamination with thromboplastic substance. This point is well illustrated by an observation on the blood of one of Howell's¹ patients: the coagulation time of 2 c.c. of blood with which, owing to defective technic, some tissue juice was mixed, was 10 minutes, whereas the same amount of blood obtained at the same time directly from a vein had a coagulation time between four and five hours.

2. *Retraction of the Clot and Enumeration of Blood Platelets.*—It is well known that normally a blood clot quickly retracts from the

10. Morawitz, P., and Bierich, R.: Arch. f. exper. Path. u. Pharmacol., 1907, lvi, 115.

11. According to Duke (Jour. Am. Med. Assn., 1910, lv, 1185) the bleeding time is the tendency to bleed from a fresh cut. The duration of such a hemorrhage is determined by blotting up on filter paper all the blood which flows from a small incision at intervals of thirty seconds. Each drop will give, in a rough way, the volume of blood shed in the given time interval.

sides of the vessel in which it is contained and expresses serum. This phenomenon has been ascribed to the presence of the blood platelets. A deficiency in the number of these elements and nonretractility of the clot has been found to be a constant characteristic of some hemorrhagic conditions, notably purpura hemorrhagica. In both of these respects, most observers have reported hemophilic blood to be perfectly normal, but since observations on the character of clot retraction may be made during a determination of the coagulation time, and since the enumeration of blood platelets is not difficult, a record of both of these factors should be made, if possible, in all complete studies of the blood in hemorrhagic conditions.

The presence or absence of clot retraction is determined simply by incubating the clot at 37.5 C. for from twelve to twenty-four hours. In normal blood retraction begins after several hours, and is complete within eighteen to twenty-four hours; in pathologic blood, on the contrary, contraction may occur only after a long time or not at all.

For the enumeration of blood platelets a number of methods are available, but that of Wright and Kinnicutt¹² appears to have given the most reliable results in the hands of most workers. It has been our experience that successful counts can be obtained only with freshly prepared solutions and with perfectly clean apparatus.

3. *Quantitation of the Fibrin Factors—Method of Notation.*—The recorded observations include only determinations of prothrombin, antithrombin and fibrinogen. There is little of positive evidence in favor of the view that a deficiency of calcium exists in hemophilic blood (Morawitz and Lossen,⁷ Nolf,⁸ Addis,⁹ and Hess¹³). A consideration of this factor, therefore, may be omitted in this study. According to the best experimental proof the existence of a thrombokinase in the sense of Morawitz is hypothetical. What is usually designated as thrombokinase in the blood and other tissues is the coagulation accelerating substance known under the terms of zymoplastic (Schmidt) or thromboplastic substance (Nolf and Howell). Whether or not this material is deficient in hemophilic plasma cannot be determined with any certainty because no direct method of studying this factor is available. Some information concerning this substance as it exists in the blood can be obtained by an enumeration of the platelets; for these elements, as will be recalled, are an important source of thromboplastic substance. But information obtained from this source is incomplete, inasmuch as all of the formed elements of the blood have been shown to possess thromboplastic properties to a greater or less extent.

12. Wright, J. H., and Kinnicutt, R.: A New Method of Counting the Blood Platelets, *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

13. Hess, A. F.: *Bull. Johns Hopkins Hosp.*, 1915, xxvi, 372.

Prothrombin and Antithrombin.—The methods developed by Howell for the determination of prothrombin and antithrombin have been reviewed extensively in recent literature (Howell,¹³ Drinker and Hurwitz¹⁴), and only brief reference to them will be made in this paper. The prothrombin deficiency which forms such a distinguishing feature of hemophilic blood, in contrast with other bloods, is demonstrated very clearly if to the specimen of clear plasma obtained by first oxalating and centrifugalizing the blood, calcium chlorid is added in optimum concentration. A specimen of normal blood so treated shows usually a coagulation time as measured by the invertibility of the clot of about ten minutes, whereas, the clotting time of hemophilic blood, treated in a similar manner, may be delayed from one to four or five hours.

Protocol 1 gives the results of one of the comparisons made between hemophilic and normal blood (Table 1), and shows the manner in which the reaction is carried out in practice.

PROTOCOL 1.—PROTHROMBIN TEST (CASE 3, NOV. 1, 1915)

Oxalated Plasma, Drops	CaCl ₂ 0.5 Per Cent., Drops	Coagulation Time	
		Patient	Control
5	1	Clot in 24 hours... ..	14 minutes
5	2	Partly clotted in 3 hours	12 minutes
5	3	Clot in 180 minutes.....	14 minutes
5	4	Partly clotted in 3 hours	14 minutes

The control specimen of blood in this particular experiment, it will be noted, clotted in twelve minutes. In order to indicate the extent of the delay in coagulation shown by the recalcified plasma, the term prothrombin quotient has been introduced. This is obtained by dividing the coagulation time of the specimen of pathologic plasma containing the optimum amount of calcium by the coagulation time of the normal blood tested at the same time and by the same method. In the present instance the prothrombin quotient is 180 divided by 12 or 15. This indicates in a rough way that in this particular experiment the coagulation time of hemophilic blood was fifteen times that of normal blood. In subsequent paragraphs it will be shown that this figure, however, has no especial clinical significance inasmuch as the gravity of the hemophilic condition has not been found proportional to the length of time required for the plasma to clot.

14. Drinker, C. K., and Hurwitz, S. H.: The Factors of Coagulation in Primary Pernicious Anemia, *THE ARCHIVES INT. MED.*, 1915, xv, 733.

The method of determining the amount of antithrombin in hemophilic and in normal blood may likewise be rendered clearer by a specific example (Protocol 2).

PROTOCOL 2.—ANTITHROMBIN TEST¹⁵

Heated Plasma, Drops	Thrombin, Drops	Time Interval, Minutes	Fibrinogen, Drops	Coagulation Time, Minutes	
				Patient	Control
1	2	15	7	23	19
1	3	15	7	15	13
1	4	15	7	11	12
1	5	15	7	8	8
				57	52

$$\text{ANTITHROMBIN QUOTIENT} = \frac{57}{52} = 1.1$$

The use of the term antithrombin factor was suggested by Denny and Minot¹⁶ to make the results obtained at different times and with different reagents comparable; but the term antithrombin quotient appears to us to be more appropriate. This quotient is obtained as shown above by dividing the average of a series of suitable determinations of the pathological blood by a similar figure obtained for a control specimen. It is obvious that if the antithrombin content of the patient's blood and that of a control are the same, the antithrombin quotient will be about unity. Slight fluctuations above or below one are within the experimental error and may be regarded as normal.

Fibrinogen.—Determinations of fibrinogen were made by the heat coagulation method described in a previous communication.¹⁷ We are aware that this method of estimating fibrinogen is not beyond criticism, and that even normal individuals may show considerable variation in the amounts of fibrinogen determined in this way. In man, however, these fluctuations are not so great as in healthy animals. For clinical studies, therefore, this method is sufficiently accurate, especially if it be kept in mind that variations in the amounts of fibrinogen in disease in order to be of real significance should be of considerable magnitude.

4. *Quantitation of Serum Proteins.*—The availability of a simple and accurate method made possible a study of the different serum proteins in each of the five patients under observation. For this purpose the microrefractometric method recently described by Robertson¹⁸ was

15. The solutions of thrombin and fibrinogen necessary for carrying out this test were prepared according to Howell's method (Howell, W. H., *Am. Jour. Physiol.*, 1913, xxxii, 264).

16. Denny, G. P., and Minot, G. R.: *Am. Jour. Physiol.*, 1915, xxxviii, 233.

17. Whipple, G. H., and Hurwitz, S. H.: *Jour. Exper. Med.*, 1911, xiii, 136.

18. Robertson, T. B.: *Jour. Biol. Chem.*, 1915, xxii, 233.

found very useful. The hemophilic blood is allowed to clot spontaneously or else coagulation is hastened by shaking with glass beads, and determinations are then made of the serum albumin, serum globulin, and total protein. The purpose of such a study would be to detect the presence of any abnormality in the protein partition products, should any arise during the clotting of pathological blood. So far as the authors are aware, this phase of the study of hemophilic blood has received no attention from previous workers.

5. *Fibrinolysis*.—The possible occurrence of fibrinolytic ferments in the blood of patients exhibiting hemorrhagic tendencies has been entertained by several observers. Such ferments have been demonstrated in some intoxications in animals and in man in certain types of leukemia and in hepatic insufficiency.¹⁹ Morawitz and Lössen,⁷ who studied this point in hemophilia, found no indication of fibrinolytic activity.

The test is easily carried out, and we believe should be done as a routine on every specimen of pathological blood examined. It is simply necessary to incubate the clot at body temperature, and to observe whether any dissolution occurs in the stated time interval. Since the clot from any specimen of blood will undergo a certain amount of dissolution if left in its serum a sufficient length of time, it is well to restrict the term pathological fibrinolysis to instances of complete clot dissolution occurring within twelve hours when the blood is kept at body temperature.

CLINICAL RECORDS AND BLOOD EXAMINATIONS

In a thorough analytical study of the hereditary aspects of hemophilia, Bulloch and Fildes²⁰ conclude that instances of probable hemophilia without demonstrated inheritance are comparatively few. It is their opinion that the introduction of the "de novo" concept is based on the inability of authors to demonstrate the line of inheritance in their cases. When it is remembered, however, that the other bleeders necessary to establish the inheritance of the condition may not be members of the same family, but of collateral branches, it is clear that the hereditary characteristics can be proved only with great difficulty.

It is fair to state that cases arising spontaneously or "de novo" without a history of bleeding in the family, have been recorded,²¹ and

19. Goodpasture, E. W.: Bull. Johns Hopkins Hosp., 1914, xxv, 330.

20. Bulloch, W., and Fildes, P.: Eugenic Lab. Memoirs, Univ. of London, 1911, xii, 169.

21. Wright reports four cases without a hemophilic ancestry (Ref. 23, p. 926); Bulloch and Fildes give at length the history of a hemophilic family studied by Gettings, who was unable to find any instances of the disease among the collaterals, although these were widely inquired into over a number of generations (Ref. 20, pp. 191 and 343). In four of Schloessmann's (See Ref. 29) seven patients no history of heredity could be demonstrated, and this was true of one of Howell's patients.

that perhaps too much stress has been laid on the hereditary features. In the patients observed by us no definite history of hereditary transmission could be obtained, notwithstanding care in questioning, although it is not unlikely that the bleeding tendency existed somewhere in the direct ascendants or in the collateral branches of these families. But it is not justifiable to exclude such cases from the group of hemophilia, because there is no evidence of a similar condition in the family records. As has been shown by Howell, hemophilia is characterized by certain properties of the blood which distinguish it from other hemorrhagic diatheses. These, we believe, furnish a convenient method of making a diagnosis of hemophilia even in the absence of any proved history of the existence of the disease in the patient's antecedents.

CASE 1.—Clinical History.—(Hospital No. 6305). George R., aged 6 years. American born, of Swedish parents, was admitted to the University of California Hospital first in October, 1913, complaining of profuse and frequent nosebleed. Since then the patient has received treatment in this hospital at four different times (Hospital Nos. 6867, 8035, 9355, and 9572), each time for excessive hemorrhages associated with his hemophilic condition.

The patient's father was living and well, his mother was a healthy woman 44 years of age. According to her account she had had frequent attacks of epistaxis, and when 24 years of age had a profuse hemorrhage following the extraction of a tooth. She had noted that "black and blue" spots appeared occasionally on different parts of her body following slight trauma. Concerning the patient's ascendants, no definite information could be obtained; nothing was known of the paternal or maternal grandparents. The patient's aunt had four or five boys who were apparently free of any hemophilic taint. The patient had one sister 9 years of age who was in perfect health, and had at no time shown any signs of a hemophilic tendency, and, according to studies of her blood, there was no abnormality existing at the present time (M. R., Table 3). Two children had died when a few days old. The cause of their death was not known to the mother.

The patient was a full-term child; his development had been normal. The most important feature of his past history was an attack of pertussis at the age of $2\frac{1}{2}$ years. The frequent hemorrhages from the nose, which form such a persistent symptom of his present condition, dated from this infection. In September, 1913, several weeks previous to his first admission for epistaxis, the patient cut his hand and bled for four days. Bleeding from the nose continued at intervals during his first four months' stay in the hospital. At times the hemorrhages were very severe and various forms of treatment were tried: two direct transfusions with little immediate or permanent effect on his condition; and the injection of human and rabbit's blood serum and of rabbit's whole blood both subcutaneously and locally to the bleeding points.

The patient was again admitted for profuse epistaxis twenty-two days following his discharge from the hospital. Two days after entrance he had a profuse hemorrhage from the stomach, and for the next few days passed tarry stools. So far as could be learned, this was the first sign of the gastro-intestinal hemorrhages which had occurred also during his last admission to the hospital. The treatment this time consisted simply of local applications of horse serum to the nasal mucosa. After a four months' stay (January, 1914, to May, 1914) the patient again returned in October, 1914, because of bleeding from a wound in the chin. In June, 1915, the occurrence of nosebleed and vomiting of blood necessitated the patient's readmission to the hospital. The gastro-intestinal hemorrhages, which had appeared first in January, 1914, had now recurred.

Examination and Clinical Course.—The positive physical findings were essentially the same during each admission: extreme yellow pigmentation of the skin, sclerae, and mucosae; a loud blowing systolic murmur and a palpable liver and spleen. During his first admission, a few small petechial hemorrhages were noted over the skin of the chest, abdomen and thigh; and during his last admission a note was made of small purpuric areas over both legs and of a similar area of large size about the left elbow.

This time the patient remained in the hospital about three months. On the first and second days following his admission, he received two subcutaneous injections of his father's blood, one of 6 c.c. and one of 20 c.c. Bleeding from the nose subsided, and the hemoglobin rose gradually from 15 to 40 per cent. (Dare). For a period of seventeen days (June 14 to June 30) the patient was fed on 0.1 gm. of kephalin daily.²² This was supplemented by three subcutaneous injections of 0.05 gm. of kephalin. These injections caused a rise in temperature, and small areas of redness, infiltration, and local tenderness. From August 1 to 25 kephalin in doses of 0.1 gm. was again administered by mouth. This therapy was continued with the hope of modifying the coagulability of the blood, although the patient, at the time the treatment was undertaken, had no severe attacks of bleeding.

CLINICAL SUMMARY AND BLOOD EXAMINATIONS

Certain points in the history of this patient deserve special mention: first, the absence of a definite history of bleeding in the family; second, the appearance of the first signs of uncontrollable hemorrhage at the age of 2½ years following an attack of whooping cough, and their persistence with intermissions for a period of five years. In this connection it is of interest that the hemorrhages had been confined with few exceptions to the nasal mucosa, and that trauma had been a factor in their causation only twice; third, the absence of hemarthroses and articular effusions. This is in striking contrast to the clinical histories of Cases 2 and 3, which will be discussed later.

At different times during the patient's stay in the hospital and for a period following his discharge from the hospital, studies were made of the factors of coagulation and of the formed elements. For purposes of brevity, the separate protocols have been omitted and the results presented in Table 1. The first observations on the blood coagulability were made June 11, five days after a persistent nasal hemorrhage. The coagulation time was found markedly delayed, 335 minutes as compared with thirty-two minutes for a normal control. Five days previously the patient had received several small injections of whole blood, which apparently had not influenced his condition. The prothrombin, as will be noted from the table, showed the deficiency characteristic of this disease; the coagulation time of the recalcified plasma being about nine times as great as the control. Antithrombin was present in very slight excess. A second observation made September 2 following an interhemorrhagic period of two months, showed the same marked delay in coagulation, although to a less extent; but, as

22. The kephalin used in these studies was prepared according to the method outlined by Howell (*Am. Jour. Physiol.*, 1912-1913, xxxi, 1).

will be noted, the deficiency in prothrombin was found to be actually more marked. This observation illustrates clearly that the extent of the delay in coagulation is demonstrated with greater accuracy if the blood is first oxalated and then recalcified. The antithrombin at this time was present in normal amount, and remained uninfluenced by the administration of kephalin during the months of July and August.

Six weeks after the patient's discharge from the hospital another examination of the blood was made (October 18). This observation is of extreme interest, because the blood obtained with the usual precautions and without admixture with tissue juices showed a more rapid coagulation time and a less striking prothrombin deficiency. Antithrombin was present in normal amount and the fibrinogen was slightly increased. It is difficult to explain this great increase in the coagulability of the blood. A similar improvement was noted also in Case 5, to which reference will be made later. It would appear that coincident with the waves of improvement observed clinically in these patients, there may also be some alteration in the coagulability of the blood. This improvement, however, was temporary, for two final observations, one on November 10 and another December 15, exhibited the same delay in coagulation and the same prothrombin deficiency as were recorded in June. The prothrombin observations in this patient showed that this factor is diminished in the interhemorrhagic interval as well as during the period of active hemorrhage. The observations indicate, however, that this deficiency in prothrombin may be less striking immediately following a period of profuse bleeding.

From the tabulated results, it is apparent that the retractility of the blood in this instance was normal. This is well in keeping with the persistence of a normal platelet count throughout the period of observation. These elements had been followed in this patient for over a year (Table 4) but, with two exceptions, they had been found normal or above normal. The table contains also observations on the number of white corpuscles and the relative percentage of each found in over twenty-five counts. It will be seen that the average number of leukocytes in this series was 2,150 and that of these 53 per cent. were polymorphonuclear elements. This is in harmony with the observations of Wright²³ and of Sahli,⁶ both of whom found a subnormal number of leukocytes and a smaller percentage of polymorphonuclear cells in hemophilic blood.

No fibrinolytic activity was noted in the blood of this patient, and an analysis of the serum expressed from the clot after its slow formation showed a normal percentage of serum albumin and of serum globulin.

23. Wright, A. E.: Allbutt's System of Medicine, 1909, v, 935.

CASE 2.—*Clinical History*.—(Hospital Nos. 10055 and 10343). James McC., aged 6 years, was admitted to the University of California Hospital, October, 1915. Three days before admission the patient fell and struck his left eye. The wound thus received was still bleeding on admission.

Although the parents were carefully questioned, no history of any hemophilic tendency in the immediate ascendants could be obtained. The paternal grandfather was 78 years old and in good health; the paternal grandmother died of cancer at the age of 48 years. The maternal grandmother was 71 years

TABLE 1.—SUMMARY OF BLOOD

Case	Date	Hydrogen Ion Conc. P H*	Coagulation Time, Minutes		Retrac- tibility of Clot	Fibrino- lysis	Platelets (Thrombo- plastin)
			Patient	Control			
Case 1, G. R.	9/11	355	37	+	0	352,000
	9/1	7.8	195	15	+	0
	10/13	23	16	-	0	296,000
	11/10	7.75	240	19	+	0
	12/15	220	30	-	0
Case 2, J. McC.	10/8	49	16	-	0	580,000 (Oct. 12)
	10/18	7.65	240	19	-	0
	10/21	+	...	192,000
	10/28	180	30	+	0
	11/5	7.7	120	20	+	0
	12/3	180	15	+	0
	12/8	170	15	-	0
	12/15	138	30	+	0
	12/20	210	30	+	0
Case 3, D. McC.	10/11	225	20	+	0
	11/1	7.75	240	18	+	0
	12/1	180	15	+	0
	12/8	240	15	+	0
	12/15	7.7	145	30	+	0	160,000
	12/20	240	30	+	0

* The reaction of the blood was determined colorimetrically by the method proposed by Levy, Rowntree and Marriott (*THE ARCHIVES INT. MED.*, 1915, xvi, 389).

of age; she had two brothers and five sisters, who were well. So far as could be learned, the sister's children were well, none having been troubled with bleeding. The patient's mother was of a family of two brothers and three sisters. The brothers were well. The one sister had two boys, both of whom were well.

The hemophilic tendency was first noted by the mother when the child was 3 months old. At that time she had noticed the appearance of "black and blue" swellings over the soles of the feet and the backs of the elbows following the slightest trauma. When 2 months old the child fell and struck his head. A large hematoma formed, which was opened. Bleeding from the wound continued for about two months and left the child extremely exsanguinated. At the age of 17 months the patient nicked his gum by the upper central

incisors and bled for a period of two weeks. At 2½ years of age, and again when 4 years of age, severe attacks of bleeding followed injuries above the right eye.

Joint effusions occurred repeatedly; the slightest trauma to an exposed joint having been followed by a painful swelling with areas of discoloration. The knees had suffered more than any other joints, and as a result of the repeated attacks these joints had become somewhat deformed, and showed considerable limitation of motion.

EXAMINATIONS IN CASES 1, 2 AND 3

Prothrombin			Antithrombin			Fibrinogen in Gm. per 100 c.c.	Treatment
Patient	Control	Quotient	Patient	Control	Quotient		
76	8	9.5	44	40	1.1	Ten cubic centimeters whole blood given subcutaneously June 5, and 20 c.c. June 6. Kephalin 5 c.c. (0.05 gm.) subcutaneously on July 1, 4 and 7. Elevation of temperature to 40 C. and local reaction. Kephalin 0.1 gm. doses daily from August 1 to August 25.
189	7	25 +	36	49	0.9	
57	10	5.7	40	47	0.8	0.494	
...	
160	17	9.4	61	82	0.8	
60	14	4.3	28	20	1.4	October 7 and 8 wound packed with sterile cotton soaked in 2.5 per cent. sterile kephalin and firm pressure applied. Twelve intramuscular injections of 5 c.c. kephalin (2.5 per cent. sterile solution) from October 7 to 15, and 2 intramuscular injections of 5 c.c. kephalin (1 per cent. in 6 per cent. glucose solution) from October 16 to 27. December 1 to 4 and 8 to 11 inclusive, exposure of tibiae and femora to Roentgen rays. Dose, 5 milliamperes minutes.
195	19	19	37	47	0.8	0.420	
...	
90	17	4.7	63	67	...	0.306	
60	7	6.0	
85	15	5.6	91	38	2.3	...	December 1 to 4 and 8 to 11 inclusive, exposure of tibiae and femora to Roentgen rays. Dose, 5 milliamperes minutes. Discontinued after this time because of low platelet count and prolongation of bleeding time.
72	15	2.8	103	103	1.0	...	
115	17	6.7	50	99	0.5	...	
200	14	14	130	149	0.9	...	
150	11	12 -	44	32	1.3	...	
180	12	15	57	52	1.1	0.250	December 1 to 4 and 8 to 11 inclusive, exposure of tibiae and femora to Roentgen rays. Dose, 5 milliamperes minutes. Discontinued after this time because of low platelet count and prolongation of bleeding time.
85	10	5.6	92	38	2.3	
95	...	3.8	
127	17	7.4	116	119	1.0	...	
180	14	12	128	140	0.9	

Examination and Clinical Course.—Apart from the condition in the knee joints the local injury was the chief feature in the physical examination. The left eye was swollen and completely closed; a large area of ecchymosis extended over both eyelids and over the upper part of the left cheek. Blood was oozing from the small incised wound, which, however, was found to be fairly deep.

Soon after admission the wound was cleaned and packed with gauze saturated in a 2½ per cent. solution of kephalin, which had been freshly prepared and sterilized. Some of the kephalin mixed with sterile vaselin was made into a paste and also packed into the wound. This treatment was repeated several times, and each time firm pressure was applied. By this means the external oozing was controlled within twelve hours, but some extravasation of blood still continued into the tissues as was apparent from the discoloration which spread over the whole of the left side of the face. Within

five days, however, the swelling and ecchymosis had commenced to disappear. On the sixth day, the pack was removed from the wound without the occurrence of any bleeding. As indicated in Table 1, the local application of kephalin was supplemented by intramuscular injections: twelve injections of 5 c.c. each of a 2½ per cent. solution of kephalin in water were given from October 7 to October 15; and nine such injections of a 1 per cent. solution in 6 per cent. dextrose, from October 16 to October 27. Notwithstanding the frequent repetition of these injections, no local induration or tenderness appeared, nor did the patient show any untoward constitutional symptoms.

Three weeks after admission a slight trauma of the right ankle caused a hemarthrosis which subsided within a week, but for which the patient was readmitted several weeks later. During this second admission, the effects of suberythema doses of Roentgen ray²⁴ on the factors of coagulation was tried. Exposure of the long bones was made daily from December 1 to 4 and from December 8 to 11 with an intermission of four days. The principles underlying this mode of treatment and its value in this disease will be discussed in subsequent paragraphs.

CASE 3.—Clinical History.—(Hospital No. 10345). Daniel McC., aged 9 years, brother of James McC., was admitted in November, 1915, complaining of swelling and pain in the left knee joint.

Both brothers gave strikingly similar histories; in the younger of the two, the symptoms of the disease had also appeared early in infancy. The mother stated that when the child was 3 months old, she had noticed for the first time bluish areas of discoloration over different parts of the body subjected to the slightest injury. When 11 months old, on first attempting to walk, the child injured his right knee. This injury, although not severe, caused an effusion into the joint. From that time to the present, considerable difficulty had been experienced with bleeding from minor accidents, three very severe hemorrhages having occurred between the ages of five and seven years: two of these followed cuts over the right and left eyes, and one followed an injury to the palm of the right hand, from which oozing continued for four months. Nearly every exposed joint in the body had been the site of an effusion. At the age of 4 years a fall on the left elbow resulted in complete disability for a period of a year. When 8 years of age, another severe hemorrhage followed the loosening of an incisor tooth.

Examination and Clinical Course.—Many ecchymotic areas of varying size were present over the arms and legs. These, together with the joint manifestations, constituted the chief findings on physical examination. All of the joints were found somewhat enlarged, but the knees were principally affected. The right knee was irregular in contour, and showed some impairment of motion. The left knee was swollen and tender. A few ecchymotic areas were present above and below the patella. Roentgenograms of the left knee showed marked distention of the synovial sac and some loss in definition of the articular surfaces. The latter presented the appearance of a chronic arthritis. Five days' rest in bed and moderate immobilization of the joint caused some absorption of the effusion and a subsidence of the pain; but the effusion returned following a repetition of the trauma, once fourteen days and again twenty-four days after admission.

In this patient also a study was made of the effect of short exposures to the Roentgen ray on the coagulability of the blood. Eight exposures of 5 milliamperes minutes each were given, the interval between each four exposures being four days. At the end of this period the exposures were stopped because the reduction in the number of platelets and the prolongation of the bleeding time made it unwise to continue the treatment.

24. Exposure made at 12 inch target skin distance with Coolidge tube, 3 mm. aluminum filter, 6 inch parallel spark gap, 5 milliamperes minutes, 1 20 erythema dose. We are much indebted to Dr. H. E. Ruggles of the University of California Hospital for making the exposures.

CLINICAL SUMMARY AND BLOOD EXAMINATIONS OF CASES 2 AND 3

The clinical histories of both brothers differed in several interesting points from that of Case 1. In them the first symptom of the hemophilic condition made its appearance in early infancy. The application of trauma was for the most part the initiating cause of the hemorrhages beneath the skin, from the open wounds, and into the synovial sacs. Repeated involvement of the joints, especially the knees, was one of the most striking symptoms in both patients, and in each instance these articular lesions resulted in chronic arthritis with deformity and disability.

The blood of each of these patients showed during every examination the characteristic prothrombin deficiency. It will be noted, however, that wide fluctuations in this factor were observed from time to time. These variations may best be considered in connection with the clinical condition and the form of treatment which was carried out. The first examination of the blood in Case 2 was made four days after the onset of bleeding (October 8). As the tabulated results indicate, the coagulability of the blood during this period was markedly increased. This is the only time that such a diminution in coagulation time during an attack of hemorrhage was observed in any of the patients. Increased coagulability of the blood, on the other hand, has been noted twice during the interhemorrhagic period (Cases 1 and 5). It would seem, therefore, that the contention of Sahli¹⁰ that, during the hemorrhagic period hemophiliacs usually show a reduced or even a normal coagulation time, is not supported by our studies.

In other respects the blood of both brothers showed no abnormalities. Clot retraction was normal, and the percentages of the serum proteins and the plasma fibrinogen did not vary widely from values obtained for normal blood. With one exception, the antithrombin factor approximated one. In both patients a transitory antithrombin excess was observed about forty-eight hours following the commencement of exposure to the Roentgen rays. The effect of such exposures on the prothrombin-antithrombin balance will be discussed later.

CASE 4.—Clinical History.—Arthur S., aged 13 years, came to the dental clinic of the University of California in June, 1915, complaining of toothache. Because of his unusual history of repeated attacks of uncontrollable hemorrhage, Dr. C. R. Giles kindly referred him to us for observations on the coagulability of the blood.

So far as we could learn from the patient's mother, no bleeding tendency had existed in any members of her family. She had four sisters, all of whom had boys, but none of these had a similar trouble. She, herself, had lost one boy, aged 5 years, on account of some internal hemorrhage following an accident. But it was not possible to find out the exact circumstances attending this boy's death. It is of interest that this child was not circumcised because it was feared that the operation might result in an attack of uncontrollable bleeding.

The patient showed the first signs of the hemophilic tendency in early infancy. Circumcision performed ten days after birth was attended by profuse bleeding from the wound lasting for a number of days. From that time to the present great difficulty had been experienced with bleeding from minor accidents. When 1 year old the patient bit his tongue and bled profusely for six days. A similar accident to the gums two and a half years later again resulted in a profuse hemorrhage. This was unaffected by the application of styptics and the thermocautery. The first attack of epistaxis occurred at the age of 4 years, and since then the attacks have been frequent, especially in warm weather. Three very severe hemorrhages occurred between the ages of 6 and 10 years: the first followed an injury to the hand, which bled for over a week; the second and third attacks of bleeding were occasioned by extraction of teeth. Several months ago the patient again had four teeth extracted, and bled for seven to eight days.

CASE 5.—Clinical History.—Edward O., aged 11 months, came under observation in October, 1915. The patient was seen first by Dr. Florence M. Holsclaw, to whom we are much indebted for the opportunity of studying the blood and for the clinical notes.

TABLE 2.—SUMMARY OF BLOOD

Case	Date	Hydrogen-Ion Conc. + P H	Coagulation Time, Minutes		Retractility of Clot	Fibrinolysis
			Patient	Control		
Case 4, A. S.	6/21	...	150	23	+	0
	10/29	7.8	210	31	+	0
	11/20	7.7	150	30	+	0
Case 5, E. O.	10/26	...	305	30	+	0
	11/18	7.6	50	8	+	0
	12/15
	12/20	...	120	20	++	0

The baby was an illegitimate child, and because of this circumstance, information concerning the family history was not obtainable. The essential facts regarding the child's health since birth and the circumstances pertaining to the present illness were obtained from Dr. Holsclaw's records. From this account it was apparent that the child had been in perfect health before the onset of the present illness. A week before the baby came under observation, the nurse had noticed several "black and blue" spots, both large and small, scattered over the body. It was also noted that the child was fretful, nervous, and wakeful at night. Two days following the appearance of the ecchymoses on the body, similar ones were noticed over both cheeks, and a few days later (October 21) a large swelling involving the whole of the left cheek appeared. The skin over the swelling was discolored, tense, and shiny, and the veins over it were dilated. The interior of the mouth was dark purplish in color. There was also a bulging inside apparently due to an effusion of blood under the mucous membrane. Palpation of this swelling did not elicit pain, and there was no elevation of temperature.

The baby was taken to Lane Hospital, October 22, where the coagulation time of the blood was tested (Addis). This was only roughly estimated, since it seemed undesirable to puncture a vein or even to make a new skin puncture. Only blood oozing from a puncture in the big toe made some hours before

was available. Capillary tubes were filled with this blood, and these were used for estimating the coagulation time. In two tubes times of seventeen and twenty minutes were observed as compared with a normal of seven minutes.

During the next few days the swelling and ecchymosis of the left cheek had begun to disappear, and bleeding from the needle prick made in the big toe three days previously (October 23) was at length stopped by pressure. October 26 the child was brought to the University of California Hospital where venipuncture was done for a study of the factors of coagulation. Two similar examinations were made subsequently, one November 18 and another December 20. At the time the patient was seen last, the swelling of the cheek had almost entirely disappeared, the "black and blue" spots had faded, and the child's general condition was excellent.

EXAMINATION OF THE BLOOD

The main points derived from a study of the blood of both of these patients have been presented in Table 2. In a general way the findings in these cases correspond to those recorded in Table 1, but it may be

EXAMINATIONS IN CASES 4 AND 5

Platelets (Thrombo- plastin)	Prothrombin			Antithrombin			Fibrinogen in Gm. per 100 c.c.
	Patient	Control	Quotient	Patient	Control	Quotient	
.....	73	9	8	14	17	0.8	0.373
.....	80	16	6	43	37	1.1	0.425
352,000	120	12	10	31	28	1.1	0.390
14,000	170	19	9	50	53	1.0	...
.....	80	17	5	120	90	1.3	...
248,000
296,000	100	14	14	170	149	1.1

well to call attention to several features which deserve special consideration.

During the period of observation, the fourth patient had no attacks of bleeding, but the blood showed, nevertheless, the characteristic delay in coagulability and a marked prothrombin deficiency. All of the other elements, as will be noted, were normal. The first observation in Case 5, on the other hand, was made only a few days after the onset of hemorrhage, and while the patient was still bleeding. It will be observed that the coagulation time was markedly prolonged, and the prothrombin much reduced in amount. During a subsequent examination, however, made about three weeks later, when active bleeding had entirely ceased, great improvement was noted also in the condition of the blood; but as is characteristic of this disease, the increase in blood coagulability was only temporary, for a third observation a month later showed a return to the initial condition.

The observations on the blood platelets in this patient are of extreme interest. It will be recalled that a small skin puncture, made several days after the onset of the symptoms, bled for about three days notwithstanding the application of firm pressure. This extreme prolongation of the bleeding time is well in keeping with the low platelet count (64,000) obtained at this period. Enumeration of these elements about two months later, when all signs of acute hemorrhage had disappeared, showed them to be normal. It is justifiable to conclude, therefore, that the extreme prolongation of the bleeding time was due in this instance to a deficiency in blood platelets. None of the other patients observed showed such a marked platelet deficiency; and it would appear that its occurrence is rare in hemophilia, although reduction in the number of

TABLE 3.—RESULTS OF EXAMINATION OF BLOOD OF SISTER OF CASE 1 AND OF MOTHER AND SISTERS OF CASES 2 AND 3

Case	Member of Family	Coagulation Time, Minutes		Prothrombin			Antithrombin		
		Pa-tient	Con-trol	Pa-tient	Con-trol	Quo-tient	Pa-tient	Con-trol	Quo-tient
1	Sister (M. R.).....	23	—	9	10	0.9	17	19	0.9
2	Sister (Agnes McC.)....	23	20	12	9	1.3	58	49	1.1
	Sister (Adeline McC.)..	20	23	12	10	1.2	52	50	1.0
	Mother.....	16	19	14	10	1.4	20	25	0.8

these elements has been noted by some observers.²⁵ Of interest also is the fact that the prothrombin remained low even when the platelets had returned to a normal level. The relation of blood platelets to the origin of prothrombin will be considered in another connection.

DISCUSSION OF RESULTS

Apart from the special abnormality in the fibrin factors to which reference has been made, a study of the blood of our hemophilic patients showed that hemophilic and normal bloods differ in no other respect. According to our observations, a change in the activity of the fibrin factors is not due to any alteration in the reaction of the blood, for we have found the hydrogen-ion concentration of hemophilic blood serum to vary little from that of normal blood. There also is no abnormality in clot retractility, which constitutes the third phase of coagulation. In this respect hemophilia differs from some other hemorrhagic

25. Austin, J. H., and Pepper, O. H. P.: Experimental Observations on the Coagulation of Oxalated Plasma, with a Study of Some Cases of Purpura, *THE ARCHIVES INT. MED.*, 1913, xi, 305.

conditions, notably purpura, in which this phase of coagulation has been found abnormal. The clot in hemophilic blood is slow to form, but it possesses normal retractile power; in fact, in several instances, the retraction of the clot and the expression of serum was greater in hemophilic than in normal blood.

In still another particular, hemophilic blood has been found normal. An analysis of the serum after coagulation has shown that the percentage of serum albumin and serum globulin approximates very closely that of normal human blood. It is true, as the tabulated results show (Table 6), that the average percentage content of total proteins is somewhat diminished in hemophilic blood, and that the globulin fraction shows a slight relative excess, but these differences are too small to possess real significance. On the whole, the results indicate that no abnormality in the protein partition products arises during the clotting of hemophilic blood, and that the change observed in the fibrin factors cannot be brought into association with any alteration in the serum proteins.

As already pointed out, our observations support the view of Howell that the essential cause of the delayed coagulability of hemophilic blood is attributable to a deficiency in the circulating prothrombin. The other factors of coagulation have been followed. Gravimetric determinations of the fibrinogen content of hemophilic blood have convinced us that no abnormality exists in this element. This finding is in harmony with those of Sahli,⁶ who weighed the fibrin produced by the coagulation of hemophilic blood, and found it to be within the limits of normal variation. That no qualitative defect in this factor exists has been shown by Addis.⁷ By the addition of varying amounts of thrombin to fibrinogen prepared from hemophilic blood and from normal blood, he was able to demonstrate that the former clots as rapidly as the latter. It is possible to conclude from the available experimental evidence, therefore, that no qualitative or quantitative defect exists in the fibrinogen of hemophilic plasma.

According to our results, the amount of antithrombin in hemophilic blood also exhibits little variation from the normal. For the most part, the antithrombin quotient was only slightly above or below one. Such fluctuations as were observed may be considered to lie within the experimental error, for the quotient seldom showed variations from one greater than several tenths. It is of interest that a definite excess of antithrombin was demonstrable in the blood of two patients about forty-eight hours after the commencement of Roentgen-ray exposures (Cases 2 and 3). This rise, however, was only transitory, and was no longer present five days later. These results do not support the view held by some observers that the delayed coagulability of hemophilic blood is due to an absolute excess of antithrombin;⁸ nor are they in harmony

with the experiments presented by other workers to prove that hemophilic blood contains less of this coagulation-inhibiting substance.* On the whole, our work supports the conclusion that there is no marked difference between the absolute amount of antithrombin contained in hemophilic and in normal blood plasma, although it is clear that this

TABLE 4.—RECORD OF LEUKOCYTIC AND PLATELET COUNTS FROM OCTOBER, 1913, TO OCTOBER, 1914, IN CASE 1

Date	Platelets*	W. B. C.	Poly-morpho-nuclears, Per Cent.	Large Round, Per Cent.	Lympho-cytes, Per Cent.	Eosino-phils, Per Cent.	Remarks
10/ 9/13	280,000 (W. & K.)	3,900	63.6	3.6	31.8	1	
	211,000 (P.)	67	2	31		
10 14 13	400,000 (W. & K.)	6,400	67	6	26	1	Bleeding time 1 min. 5 sec.
10/16/13	350,000						
10 20/13	800,000	8,100	54	10	36		
10 23/13	456,000	7,000	60.4	4	35	0.6	Bleeding time 3 min. 20 sec.
11/ 4/13	324,000	5,200	59.6	4.3	36.1		
11/12/13	4,600	45	18	36		
11/13/13	6,700	59	5	34	1	
11/15/13	6,000	65	5	26		
11/17/13	416,000	6,400	63	7	30		
11/19/13	5,100	46	21	30	1	
11/21/13	3,600	49	17	34		
11/24/13	3,400	41.5	8.6	48.4	1	
12/ 1/13	7,200	47	4	47	2	
12/ 8/13	8,200	46	11	46		
12 18 13	7,000	50	10	40		
12/28/13	4,200	40	5	55		
1/ 6 14	3,400	38	4	58		
2/ 2/14	8,400	60	7.5	20		
2/13/14	372,000	6,200	54	15	26		
2 20/14	256,000	6,125	43	31	23	1	Bleeding time 5 min.
3/ 8/14	5,380	40	10	46	3	
3/20/14	184,000	7,400	55	15	28		
4/ 2/14	160,000						
10/ 9, 14	11,400	53	5	34	1	Bleeding time 4½ min.
10/18/14	10,300	65	7.5	26	2	
Average	2,150	53				

* The method of enumerating the platelets used is given wherever the exact technique employed is stated in the records. W. and K. means Wright and Kinnicutt's method, P. means Pratt's method. For the most part one or the other of these two methods was used.

substance must be present in relative excess in hemophilia in proportion to the extent of diminution of the available prothrombin.

The deficiency in the amount of prothrombin was demonstrated by the simple procedure already described of oxalating the plasma and then adding to it the optimum amount of calcium. Our studies make it possible to state with confidence that this procedure furnishes a reliable means of differentiating hemophilic blood from the blood of other hemorrhagic conditions. Although this element has been followed throughout various periods of the disease, at no time has it been found normal. It has been our experience that wide fluctuations

TABLE 5.—RECORD OF LEUKOCYTES AND PLATELETS, CASES 2 AND 3

CASE 2 (J. McC.)

Date	Platelets	W.B.C.	Poly-morpho-nuclears, Per Cent.	Large Round. Per Cent.	Lympho-cytes, Per Cent.	Eosino-phils, Per Cent.	Remarks
11/24/15	8,300	70	9	21	..	Roentgen-ray exposures commenced 12/1 and ended 12/11.
12/ 3/15	216,000	15,200	53	10	33	2	
12/13/15	11,800	74	12	24	1	
12/15/15	160,000	Bleeding time prolonged.

CASE 3 (D. McC.)

10/10/15	12,900	94	15	4	..	Roentgen-ray exposures commenced 12/1 and ended 12/11.
10/15/15	580,000	11,150	56	10	32	3	
10/22/15	192,000	70	8	21	1	
11/24/15	10,400	52	10	34	4	
12/ 3/15	13,000	53	6	46	2	
12/13/15	14,600	72	7	20	..	
12/15/15	204,000	

in the amount of prothrombin may exist during the hemorrhagic as well as during the interhemorrhagic periods of the disease, and that the degree of this defect bears no definite relationship to the severity of the clinical symptoms. This point is well illustrated by the observations in Cases 1 and 2. In both of these patients a less marked reduction of the circulating prothrombin was noted immediately after an attack of bleeding, but a similar increase in this factor has been observed also during the interhemorrhagic period. On the basis of the experimental evidence at hand, it is therefore impossible to make any definite statements concerning this point.

Although it can be stated with a fair degree of certainty that the proximate cause of the delayed coagulability is due to a diminution in prothrombin, the ultimate cause of the condition is still a matter of speculation. Some knowledge concerning this more fundamental problem can be gained from the studies on the origin of prothrombin. So far as is known at present, the circulating prothrombin of the blood plasma is furnished by the blood platelets. This fact is based on good experimental evidence. But it is difficult to bring these observations into harmony with the well-known fact that the number of blood plate-

TABLE 6.—ALBUMIN, GLOBULIN, AND NONPROTEIN IN HEMOPHILIC BLOOD SERUM

Patient	Date	Total Protein Per Cent.	Total Albumin Per Cent.	Total Globulin Per Cent.	Albumin, Per Cent. of Total Protein	Globulin, Per Cent. of Total Protein	Non- protein Constitu- ents, Per Cent.
G. R., Case 1.....	11/10	7.3	5.4	1.9	74	26	1.0
	12/15	6.6	5.1	1.5	76	24	1.2
J. McC., Case 2.....	10/28	6.9	5.2	1.7	75	25	1.1
	11/ 5	6.6	4.8	1.8	72	27	1.2
	12/ 3	6.4	5.3	1.1	82	18	1.0
D. McC., Case 3.....	10/11	7.1	5.0	2.1	70	30	1.0
	11/ 1	7.0	4.2	2.8	60	40	1.0
	12/ 3	7.0	4.5	2.5	64	36	1.0
	12/15	7.6	5.0	2.6	65	35	1.1
A. S., Case 4.....	10/29	7.2	5.3	1.9	73	27	1.0
	11/20	7.3	5.3	2.0	73	27	1.1
E. O., Case 5.....	11/18	6.7	5.9	0.8	88	12	1.0
	12/20	6.6	5.5	1.1	82	18	1.0
Averages.....	6.9	5.1	1.8	73	26	1.0
Normal human serum*	7.94	6.20	1.74	78	22	1.09

* The figures for the percentage concentration of serum albumin and serum globulin in normal human blood serum are the averages obtained by Tranter and Rowe (Jour. Am. Med. Assn., 1915, lxx, 1433) on about thirty normal serums.

lets in hemophilia is normal. In explanation of this paradox, it has been assumed that the defect in question is attributable to a functional rather than to a numerical change in these elements. This assumption has received some experimental support from the recent work of Fonio.²⁶ As this worker has shown, platelets derived from hemophilic blood are less potent in hastening the clotting of such blood than are platelets obtained from normal blood. Fonio refers this defect of hemophilic platelets to a diminished thrombozym (thromboplastin) content; but it would appear that the evidence presented does not

26. Fonio, A.: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, xxviii, 313.

establish with any certainty that this functional defect of hemophilic platelets may not be due to a diminution in their prothrombin content. For, as is well known, the solution of platelets when blood is shed facilitates clotting in two ways: first, by setting free prothrombin; and second, by liberating a thromboplastic substance which hastens coagulation by neutralizing the antithrombin present normally in the circulating blood.

Furthermore, in the solution of this paradox the possibility must be kept in mind that other factors besides the blood platelets may be concerned with the origin of prothrombin. For instance, it has been shown that although a considerable diminution in prothrombin may result from the destruction of myeloid tissue following benzol injections, no direct parallelism exists between this drop in prothrombin, the number of platelets, and the extent of bone marrow injury.²⁷ These experimental observations support the view that the maintenance of the prothrombin equilibrium of the blood depends only in part on the blood platelets. At the present time it is not possible to state what tissue or tissues besides the marrow may be concerned in the elaboration of prothrombin. Thus far experiments have given no support for or against the contention of Nolf²⁸ that liver cell activity is essential for prothrombin production.

THE EFFECT OF KEPHALIN AND OF THE ROENTGEN RAYS ON THE BLOOD IN HEMOPHILIA

Studies concerning the proximate cause of the delayed coagulability of hemophilic blood naturally suggest the idea that it might be possible to devise some means of supplying to the patient the element in which his blood is deficient. A rational therapy of this kind might be approached theoretically in one of three ways: first, by a readjustment of the prothrombin-antithrombin balance to a more normal state by the introduction of thrombin or its antecedent substance, prothrombin, directly into the circulation; second, by stimulating the tissues concerned with prothrombin elaboration to form more of this deficient factor; and lastly, by a neutralization of the relative excess of antithrombin by the injection of tissue extracts. Some attempts have already been made to modify the coagulability of the blood of healthy animals by means of some of these methods. Experiments of this nature have developed the very interesting fact that in health the blood or the body can protect itself within wide limits from the effects of such injections. For instance, Davis,²⁸ working in Howell's laboratory, has shown that solutions of pure thrombin may be introduced

27. Hurwitz, S. H., and Drinker, C. K.: *Jour. Exper. Med.*, 1915, xxi, 401.

28. Davis, D.: *Am. Jour. Physiol.*, 1911-1912, xxix, 160.

into the circulation in quantities that in the test tube would cause rapid and firm clotting and yet no harm be done. It is apparent, therefore, that a healthy animal can protect itself from the effects of substances which would endanger its life by the formation of intravascular clots. The nature of this defensive reaction consists, it is thought, in the formation within the organism of a compensatory amount of antithrombin to bind either the injected thrombin or to neutralize the tissue extracts introduced into the circulation. But we do not know with any certainty whether or not the same response can occur in pathological states in which the balance is already disturbed in the direction of an increased or decreased coagulability. Indeed, only few attempts have been made to modify by these means the blood in diseased conditions associated with a defect in the fibrin factors. Because of the meagerness of data on this point, it seemed worth while to study this phase of the subject on some of the patients under observation.

On the basis of some experiments which will be recorded in another paper, we studied the influence produced by the injections of kephalin and the effect of Roentgen irradiation on the blood in hemophilia. With regard to kephalin, preliminary experiments on healthy rabbits had shown that this substance could be introduced into the circulation either intramuscularly or intravenously without any untoward symptoms, and that the immediate effect of intravenous injections was an increase in the coagulability of the blood. This increased coagulability was only transitory, however, and was followed soon by a return of the blood to a normal state, or in a few instances by a decreased coagulability. The exposure of animals to suberythema doses of Roentgen rays, on the contrary, produced no perceptible increase in coagulability, although an examination of the blood for the separate factors disclosed a transitory rise in the circulating prothrombin. The temporary character of this reaction and its failure to decrease the coagulation time can be explained in the light of the defensive power of the organism considered in a previous paragraph. And although these experiments showed that the effect of the administration of kephalin and of exposure to the Roentgen rays produced only temporary changes in the condition of the blood, the possibility still remained that these agents might act differently in conditions like hemophilia. Accordingly we decided to try the effects of one or both of these measures on three of the patients.

In the first patient observations were made on the effect of kephalin therapy alone; whereas, both the second and third patients were treated with kephalin and with the Roentgen rays. Concerning the dosage of kephalin, the preliminary experimental work had assured us that a considerable amount of this substance could be administered intra-

venously without any untoward symptoms. In some animals as much as 10 c.c. of a 1 per cent. solution were introduced into the ear vein without ill effects. Although some workers²⁹ have investigated the coagulation accelerating effect of intravenous injections of saline extracts in hemophilia, we did not feel that such injections were entirely devoid of danger, and we have, therefore, no data to present on this point. It will be noted from the clinical records that only the oral, subcutaneous and intramuscular modes of giving kephalin were used. The first method was early abandoned because it proved ineffective, and subcutaneous injections were discarded because they were painful. Intramuscular injections, on the other hand, were not followed by any local or constitutional reaction, and these were used exclusively in the second and third patients.

In the choice of the proper dosage for the Roentgen-ray exposure, we were guided largely by following the formed elements in the blood. As is well known from the work of recent observers,³⁰ small and large doses of Roentgen rays have antagonistic effects on the blood-forming organs; the former exert a stimulating action and the latter a destructive action. For our purposes, it was determined that one-twentieth of an erythema dose applied to the long bones at the stated intervals produced the desired stimulation of the blood-forming organs as evidenced by the rise in leukocytes. In one of the patients (Case 3), a drop in the platelet count and a prolongation of the bleeding time after the eighth exposure showed that the rays were beginning to act destructively, and this served as an indication for stopping the treatment.

The results of both of these forms of therapy have been presented for the most part in the clinical histories and in the tabulated results. Only a few points deserve further consideration. In order to avoid criticism, it is desirable in the first place that judgment regarding the results of any therapeutic measure used to control hemorrhage be guided by some criteria. Apart from all other considerations, it seems fair to assume that a therapeutic measure is successful only if it causes a cessation of the bleeding, and so affects the disease process itself that the coagulability of the blood is increased. On the basis of such criteria, it is not possible to make any great claims for Roentgen ray therapy and for kephalin injections in the constitutional treatment of hemophilia. Concerning Roentgen-ray treatment, it is safe to conclude that small stimulating doses do not alter in any way the condition of the blood in hemophilia. It is not possible to say at

29. Schloessmann, H.: *Beitr. z. klin. Chir.*, 1912, lxxix, 492.

30. Heinecke, A.: *Deutsch. Ztschr. f. Chir.*, 1905, lxxviii, 196. Duke, W. W.: *Variation in the Platelet Count, Its Cause and Clinical Significance*, *Jour. Am. Med. Assn.*, 1915, lxxv, 1600.

present whether these negative results in hemophilia, in contrast with those obtained in healthy animals, are due to a lack of response on the part of the blood-forming organs or whether more prolonged and more intensive treatment is necessary to effect any changes.

Furthermore, the especial therapeutic use of kephalin has not been found to lie in its influence on the disease process, for we have not been able to show that this substance administered intramuscularly in the dosage employed by us effects any noticeable change either in the coagulability of the blood or in the prothrombin-antithrombin balance. Only once did the opportunity arise to determine the value of intramuscular injections in the control of external bleeding (Case 2). But in this instance, it will be recalled, the injections were combined with the application of kephalin to the wound surface. Because similar injections in other patients had failed of effect, we are inclined to attribute the early arrest of bleeding in this case to the local action of the kephalin. Of its great hemostatic properties we have become convinced from observations on its coagulation accelerating action on whole blood in the test tube as well as when applied locally to bleeding surfaces. And although similar properties have been claimed for other tissue extracts, we believe that its thermostability gives kephalin a decided advantage over saline or aqueous extracts in that it can be sterilized without weakening its action.³¹ It is to be hoped, therefore, that kephalin will prove of real value in the treatment of uncontrollable hemorrhages from external wounds so frequently met with in hemorrhagic conditions.

SUMMARY

1. No alteration has been observed in the reaction of hemophilic blood. In all of the patients studied, the hydrogen ion concentration of the serum showed the normal variation.

2. No abnormality exists in the third phase of coagulation: clot formation is slow, but the clot, when once formed, shows normal retractile power.

3. The percentages of serum albumin, serum globulin, and total protein of hemophilic serum do not show wide variations from the normal.

4. The essential defect of hemophilic blood, which accounts for its delayed coagulability, is a diminution of the circulating prothrombin. The other two fibrin factors, antithrombin and fibrinogen, are present in normal amounts. Wide fluctuations may be observed in the pro-

31. Howell has called attention to this property of saline or aqueous extracts. He has shown that heating such solutions precipitates the protein, together with the active phosphatid. But if the phosphatid is first extracted with ether, the residue may be dissolved in water and the solution is unaffected by boiling (Am. Jour. Physiol., 1912-1913, xxxi, 1).

thrombin content of hemophilic plasma both during the hemorrhagic and interhemorrhagic periods. No definite relationship can be shown to exist between the extent of the prothrombin deficiency and the gravity of the clinical symptoms.

5. The method suggested by Howell of first oxalating the blood and then recalcifying with an optimum amount of calcium gives a simple and reliable means of diagnosing hemophilia and of differentiating it from other hemorrhagic conditions. It is to be recommended that this test be carried out preliminary to any operation on a patient exhibiting the hemophilic tendency.

6. Oral, subcutaneous and intramuscular injections of kephalin have no effect on the disease process.

7. Kephalin applied locally to the bleeding wounds of hemophiliacs brings hemorrhage to an early arrest. Because of its great hemostatic properties and its thermostability kephalin deserves an important place in the treatment of bleeding from external wounds.

8. Suberythema doses of Roentgen rays do not influence the disease process: no alteration was noted either in the coagulability of the blood or in the prothrombin-antithrombin balance.

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF THE HYDROGEN ION CONCENTRATION OF BIOLOGICAL FLUIDS, WITH SPECIAL REFERENCE TO THE ADJUSTMENT OF BACTERIOLOGICAL CULTURE MEDIA.

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INTRODUCTION.

The continuation within the organism of certain physiologi- [16]
cal processes depends in great measure upon the character and the constancy of the reaction of the body fluids. Changes in either will be attended by serious consequences to the cells and enzymes which find the body fluids, and more especially the blood, their natural medium. The biological importance of the constancy of the reaction of the blood has been especially emphasized by the recent studies of Henderson (1). These have shown that there exists within the organism a regulatory mechanism whereby the normal reaction of the blood is permanently maintained.

Equally important is the reaction of the medium for the successful perpetuation of life outside of the organism. This is well illustrated by the well-known and intimate association between the acidity or alkalinity of solutions containing active enzymes and the rate of their activity, and also by the influence which the reaction of a bacteriological culture medium exerts upon the biological characters and the viability of bacteria. It is not unlikely, too, that the success or failure attending the cultivation of groups of cells or tissues *in vitro* depends, in no small measure, upon the reaction of the plasma; indeed, the frequent transfer of such tissues from media containing the products of their metabolism to fresh plasma prolongs their life. The proper adjustment of the reaction of a biological

[16] fluid is, therefore, of the greatest importance for the favorable progress of many physiological processes.

As will be shown later, an exact knowledge of the reaction of a medium can be gained only from a determination of its hydrogen ion concentration. It is the purpose of this paper to present a simple colorimetric method which makes it possible to determine with considerable accuracy and rapidity the hydrogen ion concentration of biological culture fluids, and to show how such media can be adjusted to any optimal concentration of ionized hydrogen.

THE REACTION OF A SOLUTION.

Recent progress in our knowledge of the physico-chemical properties of solutions has changed considerably our conception of the reaction of a solution. The importance attributed to indicators in ascertaining the reaction of a medium, and to their value in the readjustment of such a medium to the proper reaction by titration, is gradually dwindling before the extensive knowledge gained by modern physico-chemical studies. Indicators, as we now know, do not necessarily change color at the neutral point, but rather at a definite equilibrium of hydrogen and hydroxyl ions which is peculiar to each indicator, this point of change being dependent upon the chemical constitution of the indicator. It follows, therefore, that the determination of the reaction of a medium by titrating to a convenient end-point is without real significance.

According to the theory of solutions, the acidity in water is explained by the occurrence of hydrogen ions, formed from dissolved electrolytes, in excess of hydroxyl ions; neutrality is, therefore, the condition when, as in pure water, the two concentrations are equal. Because it is easier and more accurate to determine the hydrogen rather than the hydroxyl ion concentration of a solution, it has become the established usage to express its reaction in terms of hydrogen ions.

[17]

PRESENT METHODS OF ADJUSTING THE REACTION OF CULTURE MEDIA.

In most bacteriological laboratories of this country adjustment in the reaction of culture media by titration has largely

replaced all other methods. In a very recent paper Clark [17] (2) has critically reviewed the method of "titratable acidity," and has set forth in a comprehensive way the reasons why the titrimetric method in its present form is inaccurate.

The inadequacy of the method of titration is attributable chiefly to the physico-chemical properties of the available indicators. The indicator most commonly employed for the titration of media is phenolphthalein which, like most indicators possesses no true end-point, since its zone of color

TABLE I.

A COMPARISON BETWEEN THE DEGREES IN REACTION (FULLER SCALE) AND THE HYDROGEN ION CONCENTRATION OF VARIOUS MEDIA.

No.	Medium.	Degrees in Fuller Scale.	Hydrogen Ion Concentration. Value for $p\dot{H}$.
1	Plain broth	+0.8	7.3
2	Plain broth	+0.4	7.8
3	Plain broth	+0.5	7.5
4	Plain broth	Neutral	8.4
5	Plain broth	+1.0	7.7
6	Veal-infusion	+1.0	7.1
7	Veal-infusion	+0.5	8.1
8	Veal-infusion	+0.9	7.6
9	Sugar-free broth	+0.9	7.58
10	Plain broth	+0.8	6.9
11	Liebig's broth	+0.5	7.9
12	Extract	+1.0	7.2
13	Ox-heart broth	+1.1	6.9
14	Liebig's broth	+0.8	6.9
15	Plain broth	+0.8	7.1
16	Chicken broth	+1.1	7.2
17	Glycerine broth	+1.0	7.45
18	Liebig's broth	+0.8	7.0
19	Plain broth	+1.0	6.6
20	Liebig's broth	+0.8	7.0

change is broad, lying between the hydrogen ion concentration of $p\dot{H}=8.00$ and $p\dot{H}=10.00$.¹ If the tint of phenolphthalein at $p\dot{H}=8.50$ is taken as a standard end point it is possible to show that media corrected from this point to definite degrees on the Fuller scale have different hydrogen ion concentrations.

¹ The significance of the expression $p\dot{H}=p$ to designate the hydrogen ion concentration will be explained in a subsequent paragraph.

[17] This discrepancy can be demonstrated quite readily, if one chooses at random various samples of laboratory media and compares the titratable acidity with the actual hydrogen ion concentration as measured colorimetrically. Table I illustrates the results of such a comparative study of twenty different batches of media. Similar observations were made by Clark who compared the titration curves obtained in the usual way with the determinations made by the use of the hydrogen electrode.

Unfortunately the use of the hydrogen electrode which gives the most accurate knowledge of the reaction of a medium in terms of the concentration of hydrogen ions is not suitable for practical use. Besides being time-consuming² the gas chain electrometric method necessitates special training in physico-chemical technique.

Heretofore the colorimetric method has not given promise, because of the lack of a suitable indicator, and because of the presence of coloring matter in the fluids to be tested. We believe, however, that both of these difficulties have been overcome in the procedure to be described.

PRINCIPLES OF THE COLORIMETRIC METHOD.

Already brief mention has been made of the principle which underlies the use of indicators in the titrimetric method. It was pointed out that the change in color of an indicator during an ordinary titration means that the hydrogen ion concentration of the solution has attained a certain degree characteristic for that indicator. Different indicators show color changes at varying degrees of hydrogen ion concentration. For example, the color of phenolphthalein changes from colorless to red between the values $\text{pH}=8.00$ and $\text{pH}=10.00$ (1×10^{-8} and 1×10^{-10}); whereas methyl orange passes from its full acid color over into its alkaline color as the hydrogen ion concentration falls from $\text{pH}=3.0$ to $\text{pH}=4.7$ (1×10^{-3} and 4×10^{-5}). At intermediate points various shades

² McClendon (Am. Jour. Physiol., 1915, XXXVIII, 180, 186) has described a new hydrogen electrode and a direct reading potentiometer, the use of which reduced the time necessary for a determination from forty to about two or three minutes.

of color are obtained, a certain color indicating a definite hydrogen ion concentration. [17]

Through the investigations of Friedenthal (3) and Salm (4) and of Sørensen (5), we now know the range of color change of a large number of indicators. From the large group studied several have been chosen for practical use, which, because of the extent of their range of color and because of the only slight interference of proteins in the test solutions, are of particular value for studies on hydrogen ion concentration.

In carrying out the colorimetric method it is necessary to have a series of standard solutions of known hydrogen ion concentration, and an indicator exhibiting a wide range of easily distinguishable color changes at hydrogen ion concentrations approximating those of the solutions to be tested. The procedure of making the readings is then quite simple, since it is necessary only to add an equal amount of indicator both to the standard solutions, and to the test solution, and to determine which of the colors in the standard series most closely matches that of the solution tested.

STANDARD SOLUTIONS AND METHOD OF NOTATION.

The standard solutions used in the method to be described were those recommended by Levy, Rowntree and Marriott (6) for the determination of the hydrogen ion concentration of the blood. These consist of standard phosphate mixtures prepared according to the directions given by Sørensen (7). [18] Since phenolsulphonephthalein shows definite variations in quality of color with small differences in hydrogen ion concentration between $\text{pH}=6.4$ and $\text{pH}=8.4$, it was chosen by these workers as the most suitable indicator. Experience in the use of these standard solutions containing phenolsulphonephthalein has further emphasized its value as an indicator for this work, not only because of the great breadth of its color range on either side of the neutral point, but also because of the ease with which the various gradations of color can be differentiated, even in the presence of the pigments which occur in most biological fluids. Furthermore, as Sørensen (8) points out, the fact that the phthalein group of indicators is more suitable for use in the presence of proteins or their split-

[18] products makes them especially valuable in studying the reaction of biological fluids by the colorimetric method.

In recording hydrogen ion concentration it is most convenient to use logarithmic notation as employed by Sørensen (9) rather than to record the actual concentrations because the significant variation is in the logarithm of the numbers which represent the quantity of hydrogen ions. For instance, N/10 hydrochloric acid is 0.091 N with respect to its hydrogen ions, and the hydrogen ion concentration is expressed conveniently as 9.1×10^{-2} or simply $10^{-1.04}$, the index, -1.04 , being the logarithm of 0.091. The method of notation is still more simplified by dropping the 10 and minus sign and designating the hydrogen ion concentration by the expression $p\dot{H}=1.04$, where $p\dot{H}$ is the hydrogen ion exponent.

TABLE II.
EQUIVALENTS OF THE LOGARITHMIC VALUES $p\dot{H}=6.4$ TO $p\dot{H}=8.4$
IN ACTUAL CONCENTRATIONS OF IONIZED HYDROGEN.

Logarithm $p\dot{H}$.	Actual Hydrogen Ion Concentration \dot{H}	Logarithm $p\dot{H}$.	Actual Hydrogen Ion Concentration \dot{H} .
6.4	4.0×10^{-7}	7.5 ³³	0.32×10^{-7}
6.6	2.5×10^{-7}	7.6	0.25×10^{-7}
6.8	1.6×10^{-7}	7.7	0.2×10^{-7}
7.0	1.0×10^{-7}	7.8	0.16×10^{-7}
7.1	0.8×10^{-7}	8.0	0.1×10^{-7}
7.2	0.63×10^{-7}	8.2	0.063×10^{-7}
7.3	0.5×10^{-7}	8.4	0.04×10^{-7}
7.4	0.4×10^{-7}		

³³The hydrogen ion concentration of the blood averages about 0.3×10^{-7} or $p\dot{H}=7.5$.

In Table II is given the equivalent of the logarithmic notation in actual concentrations of hydrogen ions. Only such equivalents are given which fall within the range of color change for phenolsulphonephthalein.

METHOD.

1. *Method Used in Comparing Colored Fluids.*—One of the greatest obstacles met with in the application of colorimetry to the determination of the ionization of biological fluids is the turbidity and the pigment present in the majority of such

fluids. It is obvious that the addition of an indicator to a [18] solution which is already colored gives rise to a tint which cannot be matched against a standard color scale made up with distilled water. This difficulty has been overcome by various workers in different ways: Sørensen (10), for instance, recommends the addition to the standard solutions of several drops of a solution of some neutral dyestuff so as to compensate for the color of the fluid under investigation. Similarly, turbid solutions can be compared by adding to the standard solutions varying amounts of a freshly prepared suspension of barium sulphate. Such procedures, as must be quite apparent, render the method more complicated and less accurate.

Realizing that this difficulty had to be overcome before the method could be made applicable, we tried various means of freeing such solutions of their color—dialysis, adsorption, and ultra-filtration. The method of dialysis, recommended by Levy, Rowntree and Marriott (11) for determining changes in the hydrogen ion concentration of the blood was first tried. This procedure, although invaluable for blood determinations, cannot be made applicable to studies on the hydrogen ion concentration of biological fluids. As we know, these fluids contain substances, chiefly the phosphates, carbonates, and colloids, which tend to preserve the original hydrogen ion concentration of the solutions. The value of these so-called “buffers” must be determined, if such fluids are to be adjusted to any desired ionization. In tests upon the dialysate, however, the influence of these “regulators” is not fully ascertained. The use of filtration through collodion membranes, although more accurate, is more difficult and equally objectionable for the reasons just given.

The removal of coloring matter by adsorbents like charcoal and Kieselguhr was early discarded, because both of these substances contain free alkali which changes the hydrogen ion concentration.

In order to obviate all of these difficulties, we have constructed a simple device⁴ (Fig. 1), whereby the medium

⁴ After constructing our instrument we found that Walpole (*Jour. of Biol. Chem.*, 1910-1911, V. 207) has made use of the same principle for reading colored fluids. Instead of having the four test tubes arranged parallel to one another, he has placed them end on in pairs.

[18] tested serves as a background for the standard test color to which it imparts its own characteristic quality of color, thus making the color of the fluid to be tested directly comparable with the standard test solutions. This apparatus* consists of the parts pictured (Fig. 1): A block of wood about three inches long, two inches wide and one and a half inches thick

[19]

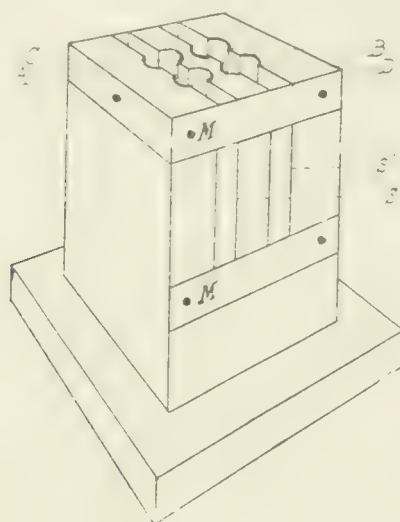


FIG. 1.—Diagram of Comparator. Into the holes *A, B, C, D*, are placed the four test tubes: *A* receives the standard comparison tube; *B* receives a tube containing the medium plus indicator; into *C* is placed the test solution to compensate for the natural color of the medium, and into *D* a test tube containing water. *S* and *S'* are the slots through which the colors are matched. *M* and *M'* are metal bands designed to equalize the portions of color fields exposed to view.

[18] serves for the construction of the comparator. Into this block four holes, *A, B, C, D*, are bored *vertically*; these are arranged in two pairs, one beside the other. Slots *S* and *S'* are then cut through the holes. If test tubes of the proper thickness are now

* Inasmuch as the test consists merely in comparing the qualities of colors and not their intensities, as is done in ordinary colorimetry, it was thought advisable to use the term comparator rather than colorimeter for the apparatus.

inserted into holes *A*, *B*, *C*, and *D*, and the apparatus is held [18] against a white background, the light in passing through each slot must pass through the two pairs of tubes.

The tube in *A* is the standard comparison tube of known [19] hydrogen ion concentration. Into the hole *B* is placed the tube containing the fluid to be tested plus the correct amount of indicator. To compensate for the color of the test fluid a sample of this without indicator is placed into *C* which is just behind the standard test color. Into *D* is placed a tube containing distilled water; this tube is used merely to make the field of view of both slots of the system similar.

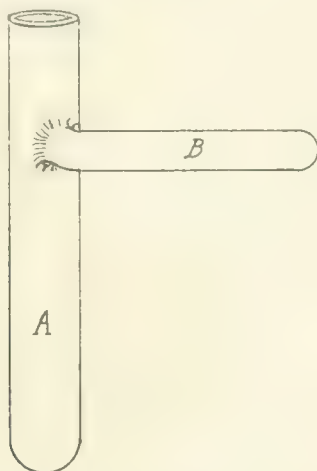


FIG. 2.—Diagram of Titration Tube. Test solution and acid or alkali are mixed in part *A*; comparisons of the color of the solution are made in part *B*, which is inserted into hole *B* of the comparator. Tube *B* is offset from part *A*, in order that part *A* will not crowd the other tubes in the comparator.

The titrations are most conveniently carried out in a specially constructed tube shown in Fig. 2. The acid or alkali used for the titrations is run into the larger tube and thoroughly mixed with the test fluid. For making the comparisons, the fluid is run into the side tube, which is of the same internal diameter and thickness as tubes *A*, *C*, and *D*; this tube is inserted into the hole *B* of the comparator.

[19] In making a reading that standard tube is chosen which at first glance appears most nearly like the color of the fluid tested. Then comparisons are made with the standard just above or below in the scale, until the closest approximation is obtained. In this way it is possible to read quite accurately between any two parts on the standard scale. It has been our experience that this method of making readings is applicable to most of the biological fluids possessing any great transparency and whose natural color is not too intense. For determinations upon yellow and straw-colored fluids it is especially useful, since phenolsulphonephthalein, in the presence of an alkali, imparts to them a brownish red tint, which can be readily matched by blending the natural color of the medium with that of the appropriate standard solution, as is done in the comparator.

2. *Preparation of the Reagents.*—In addition to the indicator N/20 acid and alkali are needed for standardization and N/1 acid and alkali for adjusting the medium to the desired concentration of ionized hydrogen.

The same strength of indicator is used in the titrations as was added originally to the standard test solutions. This is an aqueous 0.01 per cent solution of phenolsulphonephthalein made up as follows: A measured volume of indicator is obtained from a standardized solution* (1 cc.=6 milligrams) is added to the required amount of distilled water. Of this a measured volume is taken and boiled for several minutes to expel the carbon dioxide. The solution is then made up to the original volume, so as not to alter the concentration of the indicator.

In order to keep the concentration of indicator during titration the same as its concentration in the standard comparison tubes (0.3 cc. to 3 cc. or 1 to 11), the solutions of N/20 acid and alkali used for titration are so made up that one-eleventh of their volume consists of the indicator solution. For in-

* This is the well-known standardized solution of phenolsulphonephthalein prepared by Hynson, Westcott & Company, of Baltimore, Md., for testing kidney function.

stance, two liters of such a solution^{*} are prepared according [19] to the following formula:

Acid or alkali N/10.....	1000 cc.
Indicator, 0.01 per cent.....	182 cc.
Distilled water to make.....	2000 cc.

These solutions containing the indicator are used, however, only if the method is carried out according to procedure B, described below.

The normal solutions of acid or alkali are measured, sterilized and again made up to the original volume. Adjustments in the reaction of bacteriological culture media are to be made [20] after sterilization with sterile acid or alkali for reasons which will be discussed later. For the adjustment of other culture fluids, however, no such precautions are necessary.

3. *Preliminary Test.*—The medium is tested first to ascertain what its ionization is before adjustment. This preliminary test can be carried out quickly: To 3 cc. of fluid is added 0.3 cc. of a 0.01 per cent solution of the indicator, the fluid being read directly in the comparator. In most instances the culture fluid has been roughly adjusted by the usual methods so that its reaction falls within the limits of the scale ($p\dot{H}=6.4$ to $p\dot{H}=8.4$). If the medium has not received a preliminary adjustment of reaction, it may be too acid or too alkaline to be read directly. In that event titrations are carried out in the manner to be described. From such titrations is determined the amount of acid or alkali needed to bring a liter of the medium tested from its present reaction to a desired optimal ionization.

4. *Method of Titration—Procedure A.*—It may be well, before describing the manner of carrying out the titration which was finally adopted because of its greater simplicity, to present the method as it was first tried. In procedure A the several

^{*} This solution must be protected from light, moisture, and carbon dioxide. If the vessel containing it is covered with a box and the glass tubing leading from it to the graduated pipette is surrounded with an opaque paper, the solution can be kept for a long time without fading of the indicator. The solution is protected from moisture and carbon dioxide by inserting into one opening of the stopper a tube containing potassium hydroxide and calcium chloride.

[20] steps in the titration, instead of being combined as in procedure B to be described below, are carried out separately.

To each of ten test tubes ^a of the same internal diameter and thickness as those containing the standard solutions are added 5 cc. of the medium tested. From a one cubic centimeter pipette graduated into hundredths is added N/20 acid or alkali, 0.1 cc. to the first tube, 0.2 cc. to the second and so on up to 1 cc. to the tenth tube. An amount of the indicator solution corresponding to one tenth of the whole volume is now added to each tube. The amounts of the acid or alkali and of the indicator solution added to each tube in this procedure are given in Table III. This gives a series of tubes containing

TABLE III.
AMOUNTS OF N/20 ACID OR ALKALI AND AMOUNTS OF INDICATOR
SOLUTION REQUIRED FOR EACH OF 20 TUBES TITRATED
ACCORDING TO PROCEDURE A.

No. of Tube.	1	2	3	4	5	6	7	8	9	10
N/20 acid or alkali in cc.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Amount of indicator in cc.	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40

No. of Tubes.	11	12	13	14	15	16	17	18	19	20
N/20 acid or alkali in cc.	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
Amount of indicator in cc.	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50

the medium in a graded scale of hydrogen ion concentration and comparable in quality and intensity of color with the standard comparison tubes.

From among the ten tubes is chosen the one whose color most closely approximates that of the standard solution to which it is compared. The desired ionization may lie at a

^a The titration of ten such tubes will ordinarily suffice if the medium has been roughly adjusted to begin with. In Table III are given the amounts of alkali and indicator to be added to 20 tubes should it be necessary to carry the titrations above the tenth tube.

certain point between any two of the tubes of the series, in [20] which case it is necessary to interpolate. If, for instance, tube No. 1 corresponds to the value $\text{pH}=7.3$ and tube No. 2 to $\text{pH}=7.6$, and the hydrogen ion concentration desired in $\text{pH}=7.5$, the correct amount of N/1 acid or alkali to be added will lie on the curve two-thirds of the distance between that required to obtain the ionization 7.3 and that needed to give the value $\text{pH}=7.6$.

5. *Procedure B.*—For practical use it has been found advisable to combine some of the steps in the titration so as to increase the rapidity of the method without affecting its accuracy. Instead of adding the alkali and the indicator separately to each tube, the two solutions have been combined in the manner already stated in the paragraphs on the preparation of the reagents. This solution is kept in a vessel shielded from the light and the apparatus is so arranged that the solution can be delivered directly into a graduated one-cubic-centimeter pipette, provided with a ground glass stopcock on the principle of a burette. With such an arrangement, the titration can be carried out quickly and the amounts of acid or alkali used are read directly on the pipette.

The method is further simplified because the titrations can be carried out with 3 cc. instead of 30 cc. of the medium, as in procedure A. Furthermore, greater accuracy is attained, since the readings in hundredths of a cubic centimeter can now be made directly on the pipette. This does away with the necessity of interpolation for amounts between tenths of a cubic centimeter—a process which is subject to inaccuracies. Readings on the pipette in terms of the N/20 solutions used for titration can be converted directly into N/1 acid or alkali per liter by reference to Chart I, which will be described below.

The method of making the comparisons is identical for both procedures, A and B.

6. *Method of Adjusting the Media.*—The manner of adjusting the medium to the desired hydrogen ion concentration may be made clear by a specific example. The medium which has been roughly standardized to $+0.5$ or $+1.0$ (Fuller scale) is sterilized in amounts, the volumes of which have been previously measured. This may be done in ordinary flasks, which

[20] have been graduated before use to a definite volume, so that any fluid lost by evaporation during sterilization may be made up by the addition of sterile distilled water. If it is desired, for example, to adjust such a medium to the hydrogen ion concentration of the blood, which corresponds to about $pH = 7.5$ on the scale, a 3 cc. sample is titrated, as already explained, until a color is obtained which matches 7.5 on the scale. Now the amount of N/20 alkali used is read directly on the graduated pipette. Should this reading be 0.46 cc., for instance, its equivalent in terms of N/1 alkali per liter would [21] be 9 cc. This conversion of N/20 into N/1 alkali can be made directly from Chart I, on which the N/20 solution is plotted as abscissæ and the N/1 solution as ordinates. If now 9 cc. of N/1 alkali be added to a liter of the medium, the desired hydrogen ion concentration will be obtained. Should the medium be alkaline to start with, it is, of course, necessary to titrate with N/20 acid and to adjust the medium with an equivalent amount of normal acid. For this correction the lower curve of Chart I is used.

Although the titrations are made with N/20 acid or alkali solutions in order to insure greater accuracy in measurement, it is desirable for practical purposes to make the adjustment with normal acid or alkali so as not to dilute the media too much. The conversion of the amounts of N/20 solution used into the corresponding amounts of N/1 solution required per liter is accomplished most readily and accurately by plotting a curve. Such a curve must take into account three factors: the alteration in volume due to the addition of concentrated rather than dilute alkali or acid; the differences in the dilution of the "buffers" of the medium, in the one instance by N/20 solution, and in the other by N/1 solution; and lastly, the degree of correspondence between the N/20 solution used for the titration and the N/1 solution used for adjusting the medium.

Only the first of these conditions can be satisfied by a curve plotted from theoretical calculations. In fact the use of such a curve has convinced us of its inaccuracy. In order to correct for all three of the variables, it was found necessary to plot a curve from data obtained by the titration of an average

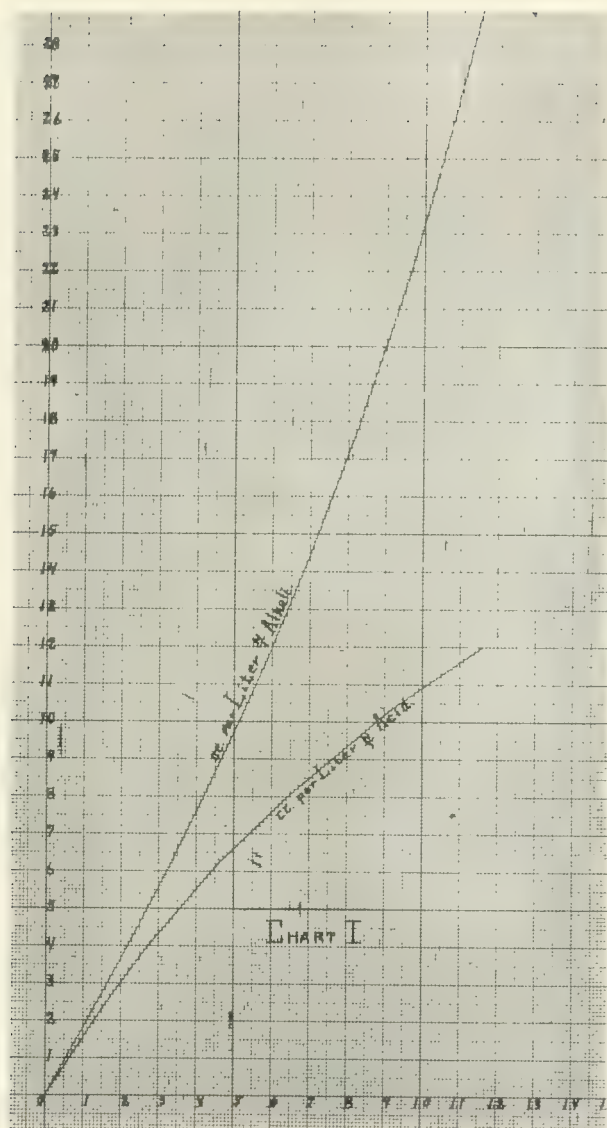


CHART I.—Curves indicating the actual amounts of N/1 acid or alkali necessary to adjust one liter of medium to the desired hydrogen ion concentration, as indicated by the amount of N/20 acid or alkali used.

[21] laboratory medium. This is done in the following manner:

To five 25 cc. samples from each of two media, one originally acid and the other alkaline in reaction, was added N/1 alkali and acid in the amounts of 0.1 cc. to the first sample, 0.2 cc. to the second and so on up to the last. From each of these samples 3 cc. were then withdrawn and to each was added 0.3 cc. of the indicator. A 3 cc. sample of the same medium was now titrated with N/20 alkali or acid, after the addition of 0.3 cc. of the indicator solution, until its color matched successively the color of each of the five tubes. The amounts of N/20 solution required were plotted as abscissæ and the corresponding amounts of N/1 solution as ordinates.

The upper and lower curves of Chart I express the relation existing between the number of cubic centimeters of N/1 alkali or acid per liter of medium and the number of cubic centimeters of N/20 alkali or acid per 3 cc. of medium necessary to bring about the same hydrogen ion concentration. In this way account is taken also of the effect upon ionization of the "buffers," by the dilution of the medium in the one instance by N/20 solution and in the other by N/1 solution. Such curves plotted for an average medium* will be found true for almost all similar media.

It is recommended that each worker using this procedure plot such reference curves. This will add greatly to the accuracy of the method, since, besides correcting for volume and dilution of "buffers," these curves correct also for any error which may arise from lack of correspondence between the N/20 solutions used for titration and the N/1 solutions used for adjusting the media.

In order that the application of this method may yield satisfactory results in the adjustment of bacteriological culture media, it is essential, in the first place, that the medium be roughly adjusted in the beginning, and secondly, that the correction in reaction be made with a sterile acid or alkali. Such a rough correction conducted in the usual manner serves

* Most of the media tested in this laboratory were prepared by extracting meat for 18-24 hours in the cold and then adding to this extract one per cent peptone, either Witte or Chapoteaut.

two-fold purpose: (1) it brings the reaction within the range [21] of the standard scale, thus making a direct reading in terms of hydrogen ion concentration possible; and (2) it necessitates the addition of smaller quantities of acid or alkali for the final adjustment of the sterile medium. This obviates the [22] dilution of the active ingredients and the possibility of precipitate formation, which may result from the addition of too large quantities of acid or alkali.¹⁰

The fallacy of correcting by the titrimetric method the reaction of a medium before sterilization applies all the more to the procedure under consideration. It is now well known that media show not only an increase in titratable acidity after sterilization (12), but, as would be anticipated, sterilization changes the equilibrium of hydrogen and hydroxyl ions (13). This change may or may not be accompanied by precipitation. It follows, that adjustments in the hydrogen ion concentration made before sterilization do not hold for media after they have been sterilized. This is all the more true of the finer adjustments in the ionization which are obtainable by the procedure outlined. It has, therefore, become the custom of some workers (14) to correct their media by adding a sterile acid or alkali to the sterilized medium.

The matter is much simplified, however, where the question of sterilization does not enter, as in the case of other culture fluids, for instance, those containing active enzymes. The latter can be readily adjusted to any optimal ionization directly, if their range of activity lies within the concentration of hydrogen ions represented by the standard solutions used.

RESULTS OF THE METHOD.

In order to determine the accuracy and the usefulness of the method, titrations were carried out on various samples of media—bouillon, agar, and gelatin, chosen at random from the different batches in the laboratory. Most of these had been adjusted previously according to the Fuller scale, so that their

¹⁰ As Clark (p. 127) points out, proteins exist in solution only within certain narrow limits of hydrogen ion concentration, and the precipitation of these and other substances of a complex medium occurs as the hydrogen ion concentration is changed.

[22] reaction fell within the limits of the colorimetric scale. In practice such media are usually found acid, necessitating, therefore, an addition of alkali to adjust them to the desired reaction. In order to test out the applicability of the method to the adjustment of media found to be too alkaline, titrations were also carried out with N/20 acid instead of alkali upon samples which had been previously made alkaline.

After determining the amount of N/1 alkali or acid needed to bring the test medium to the desired hydrogen ion concentration, this amount was added to a 25 cc. sample and a determination again made of the adjusted medium.

Forty-nine determinations were made upon twenty-three different samples of media. The results of the titrations are recorded in Tables IV and V. In Table IV are listed the various media just as they were prepared for use in the laboratory, while in Table V are recorded the results of titrations of media made alkaline for experimental purposes.

It will be seen that, for the most part, the correspondence between the hydrogen ion concentration desired and that obtained by the addition of the correct amount of alkali or acid is very close. Such accuracy in titration is readily obtained after sufficient familiarity with the technique and the standard color scale.

In several instances determinations of the hydrogen ion concentration of solid media were made. Media containing agar or gelatin were first rendered fluid by bringing them to the proper temperature. To the fluid solution was then added the indicator. The medium was inverted in the test tube several times to distribute the color evenly, and allowed to solidify.¹¹ Determinations of the hydrogen ion concentration of the solidified medium could then be made directly in the comparator. Most of the samples tested in this manner were sufficiently transparent to make a color comparison extremely accurate. In a few cases samples of agar were adjusted with the calculated amount of acid or alkali and determinations of

¹¹ Readings should be made on the solidified medium at a constant room temperature, since the hydrogen ion concentration changes with the temperature.

the adjusted samples of the solidified media were made, as in [22] the case of fluid media. These tests convinced us of the usefulness of the method for the determination of the hydrogen ion concentration of solid media.

USEFULNESS AND APPLICABILITY OF THE METHOD.

In discussing the practice of adjusting media by the method of titration in general use, attention was called to the variations in hydrogen ion concentration possessed by media adjusted to the same point on the Fuller scale. In spite of these variations in reaction, however, most bacteria have been successfully cultivated. Clark's experience has been that *B. coli* and certain streptococci will grow in media with hydrogen ion concentration values varying from $p\dot{H}=5.5$ to $p\dot{H}=9.0$. This fact is now readily explained by the presence in such media of "regulators" or "buffers." These, as already explained, prevent rapid changes in hydrogen ion concentration, which may be produced by the metabolic products of bacterial growth. This explains the value of an infusion rich in "buffers" (principally the phosphates, carbonates, and amphoteric proteins) whose ionization is little altered by a considerable variation in titratable acidity.

Chart II illustrates graphically the difference in the "buffer effect" of several such media, as measured by the amount of $N/20$ acid or alkali needed to bring each from one concentration to another given concentration of hydrogen ions. It will be observed that the media rich in "buffers" show flatter curves than those that are poor in "buffers," for the reason that a transition from one point on the scale to another requires more acid or alkali. Similar curves obtained by the use of the hydrogen electrode have been plotted by Clark (15). It is more simple, however, to obtain such curves by using the colorimetric method. Their value rests in the important information they give concerning the "buffer" content of a medium upon which depends, in large measure, its usefulness for the growth of bacteria.

The real usefulness of the colorimetric method, however, [24] must be sought in its greater accuracy and in its wide scope of

TABLE IV.

TITRATION OF Acid MEDIA SHOWING VALUE OF pH DESIRED AND THAT OBTAINED BY ADDING CORRECT VOLUME OF N. 1 ALKALI TO A 25 cc. SAMPLE OF MEDIUM.

No. of Exp.	Date.	Media.	Titration by Fuller Scale.*	Preliminary test.	Standard desired.	N. 29 alkali in cc.	N. 1 alkali added per 25 cc.	Value of pH obtained.	Remarks.
1	Aug. 6	Veal-infusion.	+1.0	Below 6.4	7.5	0.46	0.225	7.45	
2	Aug. 7	Plain broth.	+0.8	6.9	7.6	0.17	0.075	7.55	
3	Aug. 12	Liebig's broth.	+0.8	6.9	7.6	0.195	0.087	7.55	Medium poor in "buffers" as shown by titration curve.
					7.7	0.20	0.095	7.7	
4	Aug. 16	Plain broth.	+0.8	7.15	7.6	0.13	0.06	7.6	
					7.9	0.195	0.088	7.9	
					8.2	0.285	0.13	8.15	
5	—	Extract.	+1.0	6.9	7.5	0.08	0.01	7.55	Plotted as Curve V, Chart II
					7.9	0.21	0.096	7.9	medium poor in "buffers".
6	Aug. 11	Ox-heart broth.	+1.1	6.9	7.4	0.25	0.114	7.4	Plotted as Curve VI, Chart II.
					7.6	0.30	0.14	7.55	
					7.8	0.35	0.164	7.75	
7	Aug. 11	Ox-heart broth -- no peptone.	—	Below 6.4	7.2	0.36	0.17	7.15	Plotted as Curve VII, Chart II.
					7.3	0.43	0.21	7.3	
8	Aug. 16	Chicken broth.	+1.1	7.25	7.6	0.48	0.23	7.65	Plotted as Curve VIII, Chart II.
9	Aug. 17	Plain agar.	+1.0	7.1	7.9	0.26	0.12	7.9	
10	Aug. 17	Sugar-free broth.	—	7.2	7.4	0.185	0.085	7.15	
					7.6	0.26	0.12	7.55	
11	Aug. 17	Plain broth.	+1.0	6.9	7.3	0.195	0.09	7.3	
					7.7	0.275	0.13	7.65	
					7.9	0.375	0.18	7.9	
12	Aug. 17	Glycerine broth.	—	7.15	7.7	0.08	0.01	7.7	
					7.9	0.15	0.07	7.9	
13	Aug. 26	Liebig's broth.	+0.9	Below 6.4	7.0	0.285	0.13	7.05	
					7.4	0.35	0.17	7.35	
				Below 6.4	7.7	0.435	0.21	7.7	
			+1.0	6.4	7.5	0.37	0.19	7.5	

TABLE V.
TITRATION OF ALKALINE MEDIA SHOWING VALUE OF pH DESIRED AND THAT OBTAINED BY ADDING THE CORRECT VOLUME OF N 1 ACID TO A 25 CC. SAMPLE OF MEDIUM.

No. of Exp.	Date.	Medium.	Titration by Fuller Scale.	Preliminary Test.	Standard desired.	N. 20 acid in cc.	N. 1 acid added per 25 cc.	Value of pH obtained.	Remarks.
14	Aug. 30	Ox-heart broth.	—	6.9	7.3	0.17	0.08	7.25	
			—	7.0	7.8	0.285	0.13	7.8	
15	Aug. 30	Ox-heart broth plus 10% bile.	+1.0	6.9	7.6	0.30	0.15	7.55	
			—	7.4	7.4	0.25	0.12	7.35	
			—	7.1	7.7	0.21	0.1	7.65	
1	Aug. 18	Plain broth.	—	8.3	7.9 7.4	0.33 0.49	0.12 0.16	7.9 7.45	Plotted as Curve I, on Chart II.
2	Aug. 19	Sugar-free broth.	—	8.3	7.8 7.4	0.21 0.33	0.08 0.115	7.85 7.45	Plotted as Curve II, on Chart II.
3	Aug. 20	Liebig's broth.	—	7.7	7.3	0.44	0.15	7.3	Plotted as Curve III, Chart II.
4	Aug. 20	Liebig's broth —no peptone.	—	8.4	7.9 7.6 7.3	0.44 0.64 0.82	0.15 0.20 0.24	7.9 7.6 7.3	
5	Aug. 21	Ox-heart broth —no peptone.	—	—	7.5 7.1	0.33 0.52	0.12 0.17	7.5 7.1	Plotted as Curve V, Chart II.
6	Aug. 24	Ox-heart glucose broth.	—	7.6	7.1	0.10	0.04	7.1	
7	Aug. 26	Liebig's broth.	—	8.35	7.9 7.5 7.2	0.21 0.44 0.56	0.08 0.15 0.18	7.9 7.55 7.25	
8	Aug. 30	Ox-heart broth plus 10% bile.	—	8.3	7.9 7.3	0.33 0.56	0.12 0.18	7.85 7.3	

* Recorded readings by Fuller method were made before sterilization.

[21] applicability. It will be found of great value in the adjustment of the hydrogen ion concentration of media for organisms which are more sensitive to the reaction of their culture fluids. The method is comparable in a way to the fine adjustment of a microscope, the method of titratable acidity serving only to adjust media coarsely for the growth of the average organism. Its further usefulness will be found in careful studies

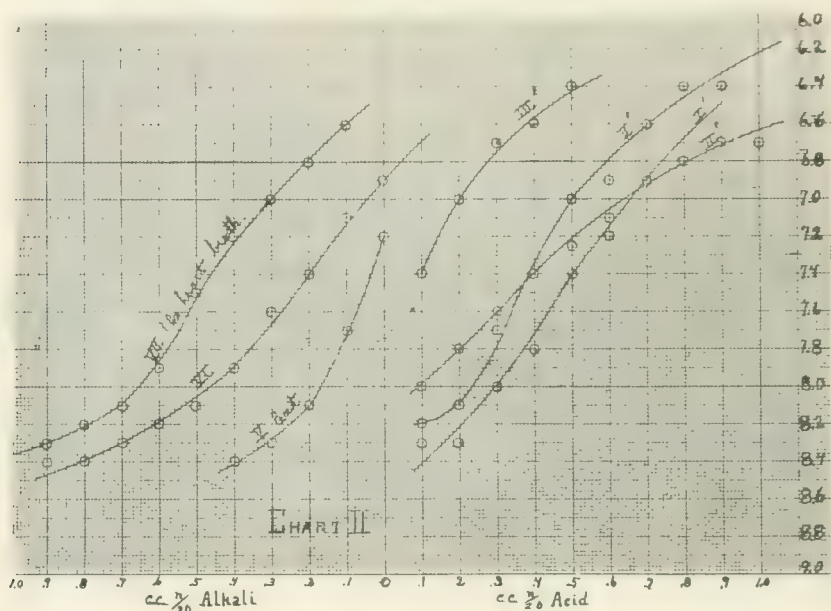


CHART II.—Titration curves of various media obtained with phenolsulphonephthalein, showing the effect of "buffers" upon hydrogen ion concentration.

upon the morphology, mutations and metabolisms of organisms, bacteria²² and also protozoa. Whether the colorimetric method in its present form is suitable as a substitute for those now in general use in bacteriological laboratories can be decided only after it has been accorded a more extensive application.

²² Blumenthal (Ztschr. f. klin., Med., 1895, XXVIII, 223) has shown that the reaction of the medium influences appreciably the relative proportions of the end-products of fermentation and putrefaction.

Already we have found the method, with some modifications, [24] useful in determining the reaction of other biological fluids. Further studies in this direction are now being carried out in this laboratory.

In conclusion, we wish to express our indebtedness to Drs. Rowntree and Marriott for providing us with a set of the standard test solutions, made under their supervision by Mr. Dunning, of Hynson, Westcott & Company, Baltimore, Md., and for valuable suggestions regarding their use.

BIBLIOGRAPHY.

1. Henderson, L. J.: *Ergeb. d. Physiol.*, 1909, VIII, 254; *Jour. of Biol. Chem.*, 1911, IX, 403.
2. Clark, W. M.: *Jour. Infect. Dis.*, 1915, XVII, 109.
3. Friedenthal, H.: *Ztschr. F. Electrochem.*, 1904, X, 113.
4. Salm, Ed.: *Ztschr. f. physik. Chem.*, 1906, LVII, 471.
5. Sørensen, S. P. L.: *Ergeb. d. Physiol.*, 1912, XII, 393.
6. Levy, E. L., Rowntree, L. G. and Marriott, W. McK.: *A Simple Method for Determining Variations in the Hydrogen Ion Concentration of the Blood.* *Arch. Int. Med.*, 1915, XVI, 389.
7. Sørensen: *Loc. cit.*, p. 432.
8. *Ibidem*, p. 445.
9. *Ibidem*, p. 401.
10. *Ibidem*, p. 426.
11. Levy, Rowntree and Marriott: *Loc. cit.*
12. Hesse: *Ztschr. f. Hyg.*, 1904, XLVI, 1.
13. Clark: *Loc. cit.*, p. 127.
14. Deeleman: *Arb. a. d. k. Gsndthsamte*, 1897, XIII, 374.
15. Clark: *Loc. cit.*, p. 121.

A LABORATORY INFECTION CAUSED BY A BOVINE STRAIN OF BACILLUS ENTERITIDIS

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Cases of meat poisoning caused by *Bacillus enteritidis* (Gärtner) or closely allied organisms are very rare in the United States. The rarity of such infections is surprising in the light of numerous observations showing that food-producing animals, as well as experimental animals, are not infrequently infected with these organisms. In recent years, for instance, paratyphoid organisms have been isolated from canary birds and horses, and *B. enteritidis* has been found present in guinea-pigs, rats, dogs (Torrey and Rahe¹), and calves (Meyer, Traum, and Roadhouse²). All of these animals act, in various ways, as sources of infection for man, and in fact the history of meat-poisoning epidemics has shown that they coincide in a most remarkable manner with the consumption of meat from animals infected with septic diseases caused, for the most part, by bacteria which biologically are identical with those isolated from the gastro-intestinal canal of cases of meat poisoning in man. Workers have always been disappointed in not being able to demonstrate experimentally the pathogenicity for men of the animal strains of the paratyphoid-enteritidis group of bacteria, altho descriptions of a few human infections with mouse typhoid bacilli³ have supplied some valuable information concerning the pathogenesis of paratyphoid organisms. In the light of the many undecided questions which such infections present for consideration, it seems worth while to give a description of an interesting laboratory infection which took place during an investigation of the etiology of infectious diarrhea of calves.

THE CASE

History.—A young man, aged 26 years, was infected January 14 while assisting in the feeding of a calf with sterilized milk containing 25 c.c. of a 24-hour-old culture of the paracolon bacillus (*B. enteritidis*, Gärtner, Strain

¹ Jour. Med. Research, 1912, 27, p. 315.

² Jour. Am. Vet. Med. Assn., 1916, 49, p. 17.

³ See the publications of Trommsdorff, München, Med. Wehnschr., 1903, 50, p. 2092; Meyer, *Ibid.*, 1905, 52, p. 2261; Shibayama, *Ibid.*, 1907, 54, p. 979; and Handson, Williams, and Klein, Brit. Med. Jour., 1908, 2, p. 1547.

1239), which had been isolated 30 days previously. The patient could not recall the manner in which he had contaminated his hands nor did he remember what precautions he had employed for disinfecting them before the evening meal.

From 10 to 12 hours later the patient was seized with severe abdominal cramps, nausea, and diarrhea. By this time he had passed from 3 to 4 semi-solid stools, and by the end of the first 20 hours he had passed 10. The nausea, abdominal cramps, and flatulence continued. The temperature at this time was 101 F., and the patient complained of loss of appetite, headache, and thirst. The tongue was coated, and there were marked fetor oris, and suppression of urine.

Forty-eight hours later the temperature fell to 99.9 F., but the patient still felt weak and depressed. Abdominal pain, tenesmus, and diarrhea continued. During the second 24 hours, he had passed 12 watery stools. These were deeply bile-stained, like rice soup in character, and contained greenish pellets of fecal matter. In the evening the patient took 5 grains of calomel. The diarrhea did not cease, the patient passing 10 more liquid stools in the subsequent 24 hours. These were light-yellow but contained very little mucus. In the evening the patient again took 5 grains of calomel.

On January 18 the patient's temperature again rose to 100 F. He felt weak and thirsty, with a peculiar desire for salt. On this day the patient had passed from 8 to 10 stools. The diarrhea persisted for 2 more days, during which he passed from 6 to 10 stools.

A week following the onset of his illness the patient felt well and ate his regular meals without any ill effect. So far as could be ascertained he had suffered from a chronic mucous colitis, which persisted more or less unaffected by the present illness, as was evidenced by the irregular elimination of mucous casts.

Bacteriologic Examination.—The relation of the gastro-intestinal disturbance to the feeding experiment was unsuspected until 4 days after the onset of the infection. At this time a solid particle of feces was enriched in bile broth and after 12 hours' incubation was plated on litmus-lactose agar. Numerous bluish transparent colonies developed, which were identified as *B. enteritidis* (Gärtner). The various subcultures obtained, behaved in every respect like Calf Strain 1239.² Its characteristic behavior in arabinose broth was noted; indeed, even the first 8 or 10 transplants failed to ferment this pentose. In the white colonies on solid arabinose endo medium there developed, in from about 5 to 7 days, red bud-like daughter colonies consisting of an arabinose "mutant."

The first few transplants of the bacillus were agglutinated only in a dilution of 1:6000 by a serum which agglutinated Strain 1239 in a dilution of 1:10,000. A sample of stool was plated on January 21 directly on litmus-lactose agar. Thirty-seven colonies of *B. enteritidis* and 30 colonies of lactose-fermenting organisms were found in 1 loopful of liquid stool. Stool samples taken on the following days gave positive findings of paracolon bacilli: January 18, 20, 21, 23, 25, 26, 28, 30, and February 1. The samples taken on February 3, 5, 10, May 10, and June 19, were found free of paracolon bacilli, even after an enrichment in bile and in malachite green broth. A large mucous cast eliminated in June proved bacteriologically to be free from *B. enteritidis*.

The pathogenicity of the strain isolated from the laboratory infection is, in some respects, interesting. Mice fed with broth directly by placing 1 drop of a 24-hour-old broth culture on the tongue, either survived or died, with all the lesions of an infection with *B. typhi-murium*, in from 8 to 15 days after

feeding of the bacteria. Mice fed however with meat soiled with a few drops of a suspension of *B. enteritidis*, succumbed regularly to the feeding infection in from 4 to 10 days, the anatomic lesions consisting of a hemorrhagic colitis, splenic tumor, and liver necroses. In a few instances some of the control mice acquired the infection from infected animals occupying the same cage.

Serologic Examination.—The serum of the patient agglutinated neither the typhoid bacillus nor the paracolon bacillus isolated from his stools 6 days after the onset of the gastro-intestinal infection.

On February 3 the serum of the patient was tested against various intestinal organisms, and agglutination occurred in a dilution of 1:1280 only with the paracolon organisms isolated from the patient and with those previously cultured from the calves. No agglutination with one typhoid bacillus strain was obtained.

TABLE 1

RESULTS OF AGGLUTINATION TESTS IN A CASE OF INFECTION WITH *B. ENTERITIDIS*

Antisera	Bacillus of Patient	<i>B. Enteritidis</i> (Strassburg)	<i>B. Enteritidis</i> (A. M. N. H.)
<i>B. typhosus</i> (Cross).....	1:200	1:40	1:60
<i>B. paratyphosus</i> A (polyvalent) ..	0	0	1:200
<i>B. paratyphosus</i> A (Strassburg).....	0	0	1:20
<i>B. paratyphosus</i> B (polyvalent, Nos. 1 and 2) ..	0	1:2000	1:1000
<i>B. paratyphosus</i> B (thoma, Strassburg) ..	0	0	0
<i>B. suipestifer</i> (Strassburg).....	0	0	1:100
<i>B. suipestifer</i> V.....	0	1:20	1:20
<i>B. suipestifer</i> (Voldagsen).....	0	0	0
<i>B. suipestifer</i> (typhi-suis).....	0	0	0
<i>B. abortus-equinarius</i>	0	0	0
<i>B. enteritidis</i> (A. M. N. H.).....	1:10,000	1:10,000	1:10,000
<i>B. enteritidis</i> (Strassburg).....	1:10,000	1:40,000	1:10,000
<i>B. enteritidis</i> (guinea-pig, Plotz No. 4).....	1:10,000		
<i>B. enteritidis</i> (No. 5, calf strain 1239).....	1:6000		
<i>B. enteritidis</i> (No. 18).....	1:2000		1:6000
<i>B. typhi-murium</i> (Loeffler No. 1).....	1:10,000	1:8000	1:10,000

Monovalent and polyvalent sera prepared with well-known organisms of the typhoid paratyphoid groups gave, with the paracolon bacillus of the patient, reactions which are summarized in Table 1. For comparison, 2 other typical strains of *B. enteritidis* are included in the table. The absence of co agglutination of the patient's strain by paratyphoid sera is interesting in many respects.

Through the careful investigations of Poels and Jensen¹ it has become established that the so-called paracolon bacilli isolated from calves suffering with infectious diarrhea are related both serologically and biochemically to *B. enteritidis* (Gärtner) and to the ratin bacillus. These findings naturally suggest the possibility that these organisms may also play a part in the etiology of certain enteric fevers in man. The meagerness of studies in this connection may be attributed to the fact that these organisms have been considered to be pathogenic only for man, and that food-producing animals have been thought to become

¹ Kolle and Wassermann's Handb. d. pathogen. Microorganismen, 1913, 6, p. 126.

only accidentally infected with such organisms. But up to within recent years the possibility of the existence of a reverse condition was not entertained. This was largely because of the failure to produce in large animals a paratyphoid-like disease by the inoculation of such organisms found pathogenic for man.

However, the facts concerning the distribution and prevalence of bacteria belonging to the paratyphoid-enteritidis group which have accumulated in the last few years, have led many hygienists to express the belief that all cases of meat poisoning are directly due to intravital contamination of the meat by such bacteria.

Furthermore, carefully conducted inquiries into recent outbreaks of meat poisoning have again failed completely to support this contention. In this connection it is remarkable that very few cases of meat poisoning are reported in the United States, where hog cholera is very prevalent. In this disease of swine, secondary invaders of the paratyphoid group are always present and are doubtless being taken into the human intestinal tract without ill effect. Again, the occurrence of infection in calves with organisms like *B. enteritidis* has been reported in California, and yet no epidemics of meat poisoning have been found traceable to veal infected with *B. enteritidis*.

This may be explained by the fact that in the United States, unlike Belgium and Germany, which, according to Sacquépée,⁵ are "*les terrains de predilection des intoxications alimentaires*," no emergency slaughtering of diseased animals is practiced, and the meat of such animals is rarely consumed. Moreover, the dangers from infected veal are reduced by the strict, tho wasteful, regulations adopted by the various states in the inspection of bob veal and meat in general. Infections from postmortem-contaminated meat are also reduced on account of the limited use of minced, uncooked, or half-cooked meat and the probable absence of a sufficiently large number of human carriers. The yearly increasing improvements in dairy inspection and pasteurization of milk lessen the possibility of infections by this channel.

Probably all these conditions are only in part responsible for the absence of infections with *B. enteritidis* in the United States. Thus far no proof has been brought forward to show with any certainty that the paracolon bacilli or *B. enteritidis* isolated from diseases of calves are always pathogenic for man and, therefore, always capable of causing meat poisoning. In the state of California paracolon-bacillus infections of calves are not rare; thus far, only one epidemic

⁵ Les empoisonnements alimentaires, 1909, p. 12.

of meat poisoning is on record (Hogan⁶), and this has not been subjected to a rigorous bacteriologic investigation.

In explanation of these facts two possibilities must be entertained: In the first place, the various strains of *B. enteritidis* may not be identical in their behavior towards man, or it is not unlikely that some predisposing factors, so far unknown, are necessary for successful infections. Secondly, it is possible that epidemics of meat poisoning caused by *B. enteritidis* or paracolon bacilli do occur, but that they are of such mild character as to be rarely brought to public attention.

The first possibility is apparently well supported by the observations of Wiemann⁷ and Rimpau,⁸ who state that veal of animals from which they had isolated paracolon bacilli had been eaten without causing the slightest harm. In support of this, Wiemann reports the following instance: On a farm, over 60 calves suffering from paracolon bacillosis were used as food either after slaughter or after death, and yet no infection resulted.

That the second explanation also has some facts to support it is shown by a statement of Christiansen.⁹ In Denmark, paracolon bacillosis of calves is one of the greatest scourges of the livestock industry, and yet extensive reports of epidemics of meat poisoning are unknown.

Referring to this point Christiansen⁹ in his article on paracolon bacillosis states:

"Even if very serious cases of poisoning are rarely substantiated—which does not mean that they rarely occur—still we have lately seen a case here of mild meat poisoning. Thus in the last few years wholesale cases of meat poisoning have occurred at the hospitals of Copenhagen; fortunately these cases have been of a mild nature in that the disease has been limited to thin purgation a few times together with stomach cramps and in the worst cases vomiting and diarrhea for a day. These cases have always occurred after the eating of a meat course, which in almost all cases, has been of veal. But this does not indicate that paracolon bacillosis caused them, for investigations in regard to the etiology of the cases are not at hand, but they show at any rate that cases of meat poisoning, especially such as are occasioned by the eating of veal, are not so rare, and like cases could very well occur in greater number in the country, without being brought to the knowledge of the public if peculiar circumstances connected with their appearance, in hospitals and like institutions, had but made them better known. It is therefore not right to say that meat poisoning rarely occurs here in this country, nor is it right to use this as an argument for the safety of man against the paracolon

⁶ Bull. California State Board of Health, 1908, 4, p. 67.

⁷ Thesis, University of Bern, 1909, p. 24.

⁸ Klin. Jahrb., 1911, 22, p. 145. Arch. f. Hyg., 1912, 76, p. 9.

⁹ Rep. Serum Lab., Roy. Vet. and Agr. High School, Copenhagen, 1915, 35, pp. 4, 73.

bacilli." (I am indebted to Miss Louise H. Madsen for the translation of the Danish text.)

The necessity of a careful bacteriologic investigation of every gastro-intestinal infection is again suggested by these observations, and the collection of all the data concerning the behavior of paracolon bacilli in man is made imperative.

DISCUSSION

The detailed description of a laboratory infection with *B. enteritidis* isolated from the blood stream of a calf suffering from infectious diarrhea demonstrates the fact that such strains can acquire pathogenicity for man. The course of the infection was severe, probably due to predisposing conditions in the intestinal tract of the patient. This predisposition in form of a mucous colitis is doubtless of considerable importance, since at least 5 other members of the laboratory staff handled, in a very careless manner, feces, organs, and cultures richly impregnated with these paracolon bacilli, and yet no additional infection was brought to our attention. Unfortunately specimens were not obtainable from all the men who handled material contaminated with paracolon bacilli. It would have been interesting to determine the possible occurrence of individuals who could temporarily eliminate the bacilli, as Conradi¹⁰ and Rimpau⁹ were able to show on several occasions. Thus, in the famous meat-poisoning epidemic of St. Johann, numerous persons who had eaten infected meat remained in perfect health and yet eliminated Gärtner bacilli, according to Rimpau. That *B. enteritidis* of animal origin is only pathogenic in man when particularly favorable conditions obtain in the internal tract for their localization, is quite clear from numerous statements and from the evidence presented; it is therefore unnecessary to assume that some strains are more virulent than others. Naturally, passage through man will enhance their pathogenic properties for man, and contact infection, in which milk and other foodstuffs can act as vehicles, will result.

It would appear from our observations that in this instance the introduction of heat-resistant toxins together with the bacteria did not play a very important part in the infection. The toxin produced in the broth was considerably diluted with milk and it is proper to assume that the bacteria alone were taken into the intestinal tract of the patient.

¹⁰ Klin. Jahrb., 1909, 21, p. 421.

According to the experiments on mice the addition of meat to the paracolon bacilli enhances the chances for a successful infection. Probably the toxins which are more extensively produced in meat than in other media, as well as the catarrhal inflammation which results from a diet of raw meat in mice, may be the predisposing factors for the infection.

The virulence of the paracolon organisms was also not particularly high for calves; one feeding experiment did not result in the death of the animal and it was noted that several calves recovered spontaneously from contact infection contracted under natural conditions.

The course of the laboratory infection corresponded well with the typical gastro-intestinal form of paratyphoid infection. Complete recovery resulted in less than 2 weeks. The causative micro-organisms disappeared entirely from the intestinal canal in 20 days after the infection. Repeated stool and numerous cast-examinations gave always negative results after February 3. This observation confirms the observations by Rimpau and others in numerous epidemics, that chronic carriers of *B. enteritidis* do not develop as the result of meat poisoning.

The positive agglutination reaction with the serum of the patient is further proof of the etiologic relation of the calf strain to the infection. The observation that the serum failed to co-agglutinate two strains of *B. typhosus* is, in the light of Langkau's¹¹ work, very suggestive. Bacteria of the Gärtner group always have the tendency to be co-agglutinated by a typhoid serum, even in high dilutions, and in contrast with sera in paratyphoid infections, sera of patients infected with *B. enteritidis* in most instances agglutinate *B. typhosus* (Rimpau¹²). Furthermore, Langkau was able to show that paracolon strains from calves are not co-agglutinated by typhoid- or paratyphoid-immune sera, a condition which is always characteristic of strains of *B. enteritidis* from man, and that this behavior gives a means of differentiating the various types of *B. enteritidis*. The agglutination tests in Table 1 apparently support his findings with the exception that a typhoid serum co-agglutinated the human as well as the calf strain (not tabulated, for the sake of brevity) of *B. enteritidis*. On the other hand, the paratyphoid and suipestifer sera did not agglutinate the paracolon strains.

¹¹ Thesis, Leipzig, 1909.

¹² München, med. Wochenschr., 1909, 56, p. 1843.

So far as we are aware, this is the first instance reported in the literature in which it has been demonstrated that a strain of *B. enteritidis* pathogenic for an animal can, in a particularly predisposed human subject, cause the typical symptoms of meat poisoning. Such susceptibility of the individual doubtless plays an important rôle under practical epidemiologic conditions of meat poisoning in transforming the strain of paracolony bacilli pathogenic for animals into one pathogenic for man, for as a rule the strains of *B. enteritidis* isolated from calves, guinea-pigs, and dogs are of low virulence and, according to a few scattered observations, do not possess the high pathogenicity for man characteristic of the true meat-poisoning organisms.

CONCLUSIONS

This paper presents clinical, bacteriologic and serologic observations of an accidental laboratory infection. A young man who apparently was predisposed to the infection on account of a chronic mucous colitis developed a severe gastro-enteritis 10 hours after having handled a bottle of sterilized milk which was artificially contaminated with a culture of *B. enteritidis* (Gärtner). The strain responsible for the infection had been isolated from the heart blood of a calf which had succumbed to infectious diarrhea. Evidence is presented to show that a recently isolated strain of *B. enteritidis* pathogenic for animals may differ from a strain pathogenic for man in its inability to be co-agglutinated by paratyphoid or suipestifer sera.

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ON THE REACTION OF THE CEREBROSPINAL FLUID*

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In its mode of origin and in its pathways of absorption, as well as in its physical and chemical properties, the cerebrospinal fluid is unique among the body fluids. Two facts derived from clinical and experimental observations have been established within recent years with a fair degree of conclusiveness, namely, the dual origin of the cerebrospinal fluid and its return to the general circulation chiefly by a process of filtration through the arachnoid villi into the great sinuses.^{1, 2} Unlike the circulating lymph, which is derived from the blood by a process of filtration, diffusion and osmosis, the cerebrospinal fluid is the secretory product of the ependymal cells which cover the choroid plexuses, although it must be admitted that certain fundamental anatomic and physiologic aspects of this problem are still unsolved.

Another interesting observation which has been brought forth by a number of workers is that these plexuses constitute a remarkably effective barrier against the entry into the cerebrospinal fluid of substances present in the circulating blood. This observation may indeed explain, in part at least, the unique physical and chemical properties of the fluid, for in these respects also it is unlike any other fluid in the body, being approached in its composition most nearly by the sweat, tears, and aqueous humor of the eye. From the circulating lymph normal cerebrospinal fluid differs in several striking particulars: it is of lower specific gravity, contains a small content of salt, only minute traces of protein, and no fibrinogen.

Concerning the reaction of the cerebrospinal fluid, little more definite is known beyond the general statement of most authors that it is alkaline. As to the degree of this alkalinity in comparison to the other body fluids and more especially to the blood little is known. According to Cavazzani—and most authors quote him—the alkalinity of the fluid is only half as great as that of the blood.^{2, 3} Mott has attempted to express the degrees of reaction in terms of percentages of

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1. Weed, Lewis H.: *Jour. Med. Research*, 1914, xxxi, 21, 51, 93.

2. Mott, F. W.: *Lancet*, London, 1910, ii, 1, 79.

3. Plaut, F.; Rehm, O., and Schottmüller, H.: *Leitfaden zur Untersuchung der Zerebrospinalflüssigkeit*, Jena, Gustav Fischer, 1913, pp. 16, 23.

sodium hydroxid. Calculated in this way, the reaction has been found by him to vary only slightly in different animals, and in man in different pathologic conditions. On an average it corresponds to 0.1 per cent. sodium hydroxid.

That such titrimetric determinations of reaction lack accuracy has been repeatedly emphasized in recent literature.^{4, 5} The more extensive knowledge gained from modern physicochemical studies has shown that the titrimetric method in its present form is inaccurate, and that an exact knowledge of the reaction of a solution can be gained only from a determination of its hydrogen ion concentration.

Although extensive studies have been made of the hydrogen ion concentration of most biologic fluids, electrometrically or by the colorimetric method, we were surprised to find almost no mention in the literature of similar determinations on the cerebrospinal fluid. Neither Sørensen⁶ nor Michaelis⁷ in their exhaustive treatises on this subject make any reference to such studies.

So far as we are aware, Polanyi, quoted by Bisgaard,⁸ was the first to determine the hydrogen ion concentration of the spinal fluid. He found the value for pH to be 10.04 (9.08×10^{-11}). Bisgaard, however, who carried out similar determinations both by the gas-chain electrometric method, as well as colorimetrically, gives the value as 8.10, although fluids obtained post mortem were found by him to be more alkaline (pH —8.46 to 9.25). This worker found also that the results obtained by the Hasselbach apparatus corresponded well with those obtained colorimetrically with phenolphthalein as indicator.

In view of the meagerness of data on this point, it seemed worth while to determine the hydrogen ion concentration of normal cerebrospinal fluid, as well as of fluid obtained from patients suffering from several types of disease. In the present paper we wish to report the results of such determinations made by the colorimetric method upon forty-seven fluids.

METHOD

Sources of Error.—In a recent communication⁵ we presented in some detail the principles and the extent of applicability of the colorimetric method for determining the hydrogen ion concentration of bio-

4. Clark, W. M.: Jour. Infect. Dis., 1915, xvii, 109.

5. Hurwitz, S. H.; Meyer, K. F., and Ostenberg, Z.: Bull. Johns Hopkins Hosp., 1916, xxvii, 16.

6. Sørensen, S. P. L.; Ergeb. d. Physiol., 1912, xii, 393.

7. Michaelis, Leonor: Die Wasserstoffionenkonzentration, Berlin, Julius Springer, 1914.

8. Bisgaard, A.: Biochem. Ztschr., 1913-1914, lviii, 1.

logic fluids, but only brief reference was then made to the sources of error of the method. These in the main are three in number,⁹ and a critical analysis of them will show that they do not materially alter the accuracy of the method in its application to cerebrospinal fluid.

1. **The Effect of Coloring Matter in Biologic Fluids:** One of the greatest obstacles met with in the use of colorimetry in the determination of the ionization of biologic fluids is the turbidity and the pigment present in the majority of such fluids. In the case of blood this difficulty was unsurmountable until Levy, Rowntree and Marriott¹⁰ suggested the method of determining the ionization of the dialysate obtained by dialyzing blood through collodion membranes for a given time interval. It is obvious that this difficulty does not arise in the case of cerebrospinal fluid, which, in health and when carefully obtained without contamination with blood, is usually a colorless, limpid fluid. Should any turbidity exist due to the presence of inflammatory products, the fluid can still be read directly by use of the comparator method described in a previous paper.

2. **Influence of Neutral Salts:** Those who have used this method are agreed that the presence of neutral salts in sufficient concentration greatly influences the point at which a given indicator changes color. In this respect various indicators show different degrees of sensitiveness. In most instances the neutral salt concentration must be two to three times that of blood in order to render the use of indicators for these determinations subject to gross error.¹¹ From the available analyses of the blood and the cerebrospinal fluid it would appear, how-

9. A source of error which should be mentioned, but which is not peculiar to any one method, is that which may result from the loss of carbon dioxid in determinations of hydrogen ion concentration on carbon-dioxid-containing fluids, such as blood and cerebrospinal fluid. The possibility of this error has been precluded in electrometric measurements by the use of a special electrode, the Hasselbach electrode, for the purpose of keeping the carbon dioxid tension at a constant level during the measurements. In the use of the colorimetric method this difficulty cannot be met with entire satisfaction, and the values for pH obtained do not in a strict sense have the absolute accuracy possessed by readings made with the hydrogen electrode. But it would appear from Mott's work² that the carbon dioxid in cerebrospinal fluid is in more stable combination than in blood, and for this reason this medium is more suited to colorimetric readings than is the blood. In every case it is important to make the determinations on perfectly fresh specimens and with a similar technic in order to give the results comparative value.

10. Levy, R. L., Rowntree, L. G., and Marriott, W. McKim: *THE ARCHIVES INT. MED.*, 1915, xvi, 389.

11. Rona, P.: *Handbuch d. Biochemische Arbeitsmethoden*, E. Abderhalden, 1911, v, 321.

ever, that the inorganic salts exist in the two fluids in about equal concentration.¹²

3. Influence of Proteins and their Split Products: Because of their colloidal properties and their amphoteric character, proteins or their split products greatly modify the point at which certain indicators change color, and this quite independently of the hydrogen ion concentration. Their presence, therefore, constitutes a source of error which at times is sufficiently great to render the colorimetric method inapplicable to solutions containing protein in high concentration. From what has already been said concerning the extremely low protein content of normal cerebrospinal fluid, it is apparent that this source of error does not apply to it. According to Mott and others, normal cerebrospinal fluid contains only 0.03 per cent. of protein, and in pathologic cases it seldom exceeds 0.25 per cent.³

From the above considerations it is clear that because of the unique chemical composition of the cerebrospinal fluid colorimetric determinations of its ionization are not subject to the same sources of error which would arise in the case of other biologic fluids.

Technic.—The principles of the colorimetric method, the preparation of the standard comparison tubes and the manner of expressing the results are recorded in recent communications.^{5, 10} In this paper it is necessary only to give the several steps in the procedure.

Spinal fluid is obtained by lumbar puncture in the usual way, and is received into thoroughly clean and dry Jena glass test tubes.¹³ Because of the clear, watery nature of the fluid and its low protein content, it is possible to make the determinations of the hydrogen ion concentration either directly or indirectly by testing the dialysate as recommended for blood by Levy, Rowntree, and Marriott.

For a direct reading 3 c.c. of spinal fluid are delivered into a small clean and dry test tube 10 by 100 mm., and 0.3 c.c. of a 0.01 per cent. solution of phenolsulphonephthalein is added. After inverting the test tube several times in order to distribute the color evenly, comparisons are made between the color obtained and those of a series of standard

12. Gautier and Zdarek (quoted by C. E. Simon, *Clinical Diagnosis*, Ed. 8, Philadelphia, Lea & Febiger, 1914, p. 501) give the concentration of chlorids, phosphates and sulphates in cerebrospinal fluid as 6.44 per mille; while Hammarsten, O. (*A Text Book of Physiological Chemistry*, Ed. 8, New York, John Wiley & Sons, 1909, p. 239) states that the blood as a whole contains in ordinary cases from 770 to 820 per mille water, with from 180 to 230 per mille solids, of which from 6 to 10 per mille are inorganic and the remainder organic solids.

13. We have found that some of the thick-walled test tubes, even when thoroughly clean, deliver up alkali in sufficient amount to render distilled water placed in them alkaline to phenolsulphonephthalein. This source of error must of course be obviated.

TABLE 1.—THE HYDROGEN ION CONCENTRATION OF NORMAL CEREBROSPINAL FLUID

Num- ber of Case	Name	Age	Diagnosis	Hospi- tal Number	Wasser- mann Count	Nonne Noguchi	Reaction of Cerebro- spinal Fluid, Value of pH	Reaction of Spinal Fluid Dialysate, Value of pH	Reac- tion of Spinal Fluid, Dialysate, Value of pH	Remarks
1	S. R.	55	Tuberculosis of wrist....	15413	B. F.	0	8.3	8.3	
2	A. H.	50	Diabetes.....	16278	B. F.	0	8.25	8.15	
3	A. C.	63	Chronic nephritis....	16295	B. F.	0	8.3	8.2	
4	C. B.	30	Paranoia	16769	B. F.	0	8.3	8.15	
5	J. G.	28	Sinushitis.....	12402	B. F.	0	8.3	8.15	
6	H. V.	7	Cerebral injury, decom- pression	House 9732	0	8.15 8.3	8.0 8.15	Three spinal punc- tures with with- drawal of consid- erable fluid
7	P. P.	53	Pulmonary tuberculosis	11107	B. F.	0	8.3	8.1	
8	W. H. T.	58	Cerebral injury....	House 9739	B. F.	0	8.15	8.05	
9	D. S.	56	Neurasthenia	14176	B. F.	0	8.25	8.2	7.5	
10	T. W.	45	Tabes (C).....	17198	B. F.	0	8.3	8.1	7.6	No positive clinical signs of tabes
11	J. S.	43	"Dizziness".....	17475	B. F.	0	8.4	8.15	7.59	
12	J. S.	54	History of syphilis....	17578	B. F.	0	8.25	8.0	7.65	No signs or symp- toms of syphilis
13	C. D.	45	Retinitis	15554	B. F.	0	8.3	7.95	No signs of syphilis
14	M. E.	20	History of syphilitic in- fection (?)	17915	B. F.	1	8.25	8.05	7.69	No signs of syphilis
15	T. F.	28	Papillitis.....	17765	B. F.	0	8.2	8.25	No clinical signs of tabes
16	A. B.	49	Tabes (C).....	B. F.	0	8.25	
17	L. F.	42	Syphilis (C).....	18178	B. F.	0	8.3	8.0	
18	A. A.	45	Tabes (C)	16983	B. F.	1	8.25	
19	T. B.	43	Syphilis (C).....	19294	B. F.	0	8.20	8.0	7.65	
Averages.....					8.26	8.11	7.66	

* B. means blood; S. F. means spinal fluid

solutions consisting of phosphate mixtures prepared according to the directions of Sørensen.¹⁴ The color in the standard series which most closely matches that of the solution tested gives the hydrogen ion concentration of the test fluid.

If it be desired to test the dialysate in order to compare its hydrogen ion concentration with the dialysate of the blood of the same patient, 1 to 2 c.c. of cerebrospinal fluid are placed into a collodion sac, which is then lowered into a small test tube containing 3 c.c. of physiologic salt solution, until the fluid on the outside of the sac is as high as that on the inside. Dialysis is allowed to continue for five minutes. The collodion sac is then removed, and 0.3 c.c. of the indicator solution is added to the dialysate. Color comparisons with the standard scale are now made as detailed above. For comparative purposes the same quantity of the same patient's blood is tested in a similar manner.

RESULTS

The Reaction of Normal Cerebrospinal Fluid.—In Table 1 have been grouped nineteen normal patients in whom the reaction of the cerebrospinal fluid was determined. These patients, as will appear, represent a large variety of clinical conditions, and in a strict sense cannot be classed as normal. But so far as the cytologic, chemical and serum tests indicate, no abnormality existed in the central nervous system, and on this ground it is justifiable to place them in the normal group.

It will be noted that in the nineteen determinations made upon the fluid directly the average value of the hydrogen ion exponent (pH) was 8.26, with a minimum value of 8.15 and a maximum value of 8.3; whereas the average of the same number of readings made on the dialysate was slightly lower, pH being equal to 8.11. Of interest also is the fact that the two lowest readings were obtained in cases of cerebral injury, treated by decompression and the withdrawal by puncture of considerable amounts of fluid. It is not unlikely that the diminished alkalinity in these instances may have been due to dilution of the fluid by hypersecretion.

As compared with the hydrogen ion concentration of the dialysate of the cerebrospinal fluid, that of the blood obtained from the same patient was found to be much higher. In the six instances in the normal group in which the blood was also tested an average value for pH of 7.66 was obtained. Thus the hydrogen ion concentration of the blood in these cases was greater than that of the spinal fluid by the value of 0.45.

14. The standard test solutions were prepared for us by Hynson, Westcott and Company, Baltimore.

In connection with the reaction of the cerebrospinal fluid in health, there are several interesting physiologic problems which still await solution. One of these concerns the relationship between the normal functional activity of the nervous centers and the reaction of the fluid. Mott² proposes the view that after normal sleep the alkalinity may be slightly greater than at the end of a day's work, because of the production of acid substances resulting from nerve cell activity.

TABLE 2.—A COMPARISON OF THE HYDROGEN ION CONCENTRATION OF BLOOD AND CEREBROSPINAL FLUID

No. of Case	Reaction of Cerebrospinal Fluid Value of pH	Reaction of Cerebrospinal Fluid Dialysate, Value of pH	Reaction of Blood Dialysate, Value of pH
	8.25	8.2	7.7
10	8.3	8.1	7.6
11	8.3	8.15	7.75
12	8.25	8.0	7.65
14	8.25	8.05	7.65
16	8.2	8.0	7.65
20	8.3	8.15	7.75
23	8.3	8.15	7.6
25	7.95	7.9	7.65
27	8.35	8.15	7.7
28	8.3	8.1	7.65
29	8.3	8.1	7.65
32	8.3	8.15	7.5
40	8.2	8.1	7.7
42	8.15	8.15	7.75
43	7.95	7.75	7.65
56	8.4	8.15	7.75
57	8.3	7.95	7.60
Average	8.07	7.66

Another problem of interest concerns the reaction of the cerebrospinal fluid in different loci. From the work of a number of observers it is now known that ventricular and subarachnoid fluids may differ in some important respects. It has been shown, for instance, that there is a slightly higher percentage of sugar in ventricular than in subarachnoid fluid, whereas the reverse is true of the albumin content; furthermore, that ventricular fluid may be richer in waste substances which may have been added to it during its passage through the sub-

arachnoid perivascular and perineuronal spaces.¹⁵ The possibility may therefore be entertained that the two fluids, which differ in these marked particulars, may also show measurable differences in reaction.

The Reaction of Cerebrospinal Fluid and of Blood Compared.—In a previous paragraph it was pointed out that blood and spinal fluid obtained from the same patient at the same time may show a considerable difference in the hydrogen ion concentration as determined by the colorimetric method. In Table 2 are given eighteen instances in which such comparative data were obtained. The average value of the hydrogen ion concentration tested upon the dialysate of the spinal fluid was 8.07 as compared with 7.66 obtained in the case of the blood dialysate. Thus the ionization of blood tested in this way is greater than that of cerebrospinal fluid by the value of 0.41. It must be emphasized, however, that the need of resorting to dialysis doubtless introduces a source of error in the data given, for the reason that the two fluids dialyzed—blood and cerebrospinal fluid—are not chemically equivalent, the former containing a higher percentage of protein and colloids, both of which are known to influence the dispersion of electrolytes through porous membranes.¹⁶ These considerations, however, do not affect the observation that the hydrogen ion concentration of the spinal fluid tested directly is lower than that of the blood.¹⁷

The Reaction of the Cerebrospinal Fluid in Disease.—In this paper the observations have been limited to those diseases in which lumbar puncture is ordinarily done as a routine measure either for diagnosis or treatment. Tables 3 and 4 include twenty-eight cases of syphilitic disease and of syphilitic affections of the nervous system in which the reaction of the spinal fluid was determined. The diagnosis in all of these patients was confirmed either by cytologic and chemical tests or by the routine Wassermann reaction of the blood and spinal fluid.

From an examination of Table 3 it will be noted that the average hydrogen ion concentration (pH) of the spinal fluid in primary and secondary cases of syphilis is equal to 8.26, while determinations of the dialysate give somewhat lower readings (pH=8.14). Similar readings made upon the dialysate of the blood in seven of these patients gave an average value of pH equal to 7.64, which is 0.5 lower than that obtained for the dialysate of the spinal fluid. By comparing these figures with those recorded in Table 1, it will be observed that these values do not differ widely from those obtained for normal fluids. The

15. Cushing, H.: Jour. Med. Research, 1914, xxxi, 12, 13, 15.

16. Ostwald, W.: A Handbook of Colloid Chemistry, Philadelphia, P. Blakiston's Sons, 1915, p. 219.

17. The hydrogen ion concentration of the blood determined by the hydrogen electrode averages 0.3×10^{-7} , or pH=7.5. The value for pH⁺ obtained by the colorimetric method is 7.66.

TABLE 3. THE HYDROGEN ION CONCENTRATION OF THE CEREBROSPINAL FLUID IN SYPHILITIC DISEASE

Number of Case	Date	Name	Age	Diagnosis	Hospital Number	Wassermann*	Cell Count	Nonne	Reaction of Cerebrospinal Fluid, Value of pH	Reaction of Spinal Fluid, Value of pH	Reaction of Blood, Value of pH
20	8/24/15	L. S.	55	Syphilis	676	B. F. S. F.	1	0	8.9	8.15	...
21	8/26/15	A. L.	50	Syphilis, Optic atrophy	1054	B. F. S. F.	1	0	8.3	8.1	...
22	9/21/15	C. B.	38	Optic atrophy	1116	B. F. S. F., H. ...	1	0	8.3	8.15	7.75
23	10/5/15	M. F.	40	Chorea	11104	B. F. S. F.	2	0	8.3	8.15	7.6
24	10/7/15	L. D.	78	Chorea	12660	B. F. S. F.	6	0	...	8.25	...
25	10/12/15	J. L.	50	Chorea	1185	B. H. S. F.	0	0	7.59	7.9	7.65
26	10/17/15	P. C.	55	Reinits	1779	B. F. S. F.	3	0	8.1	8.05	...
27	10/14/15	J. I.	50	Secondary syphilis	1814	B. F. S. F.	0	0	8.60	8.15	7.7
28	10/14/15	M. S.	...	Chorea	1814	B. F. S. F.	2	0	8.3	8.1	7.65
29	10/14/15	F. C.	L.	Chorea	1824	B. F. S. F.	56	...	8.3	8.1	7.65
30	10/31/15	L. W.	...	Mental syphilis	1855	B. F. S. F.	7	0	...	8.2	...
31	11/16/15	C. W.	50	Syphilis, Syphilitic gias (Trousseau)	1850	B. F. S. F.	1	0	8.3	8.05	...
32	11/18/15	W.	54	Chorea	18107	B. F. S. F.	0	0	8.3	8.15	7.6
33	10/15/15	C. I.	18	Secondary syphilis	1764	B. F. S. F.	173	...	8.3	8.05	...
Averages							8.26	8.14	7.61

* B. means blood; S. F. means spinal fluid.

results show, further, that in both groups the hydrogen ion concentration of the blood obtained from the same patient at the same time and similarly tested is in each instance greater than that of the spinal fluid, the average value for pH in the syphilitic cases being almost identical with that obtained in the normal cases.

Nor was any marked alteration of the normal reaction observed in the spinal fluid obtained from patients with syphilitic affections of the central nervous system, such as tabes, general paresis and cerebrospinal syphilis. The average values for pH in this group, as recorded in Table 4, are slightly lower than the normal, but the differences are too small to be of real significance. A study of the reaction of the fluid in these cases brings up an interesting point, namely, the relationship between inflammatory conditions of the meninges and the reaction of the cerebrospinal fluid. Some authors^{15, 18} entertain the view that in the presence of infection acid products are formed which render the cerebrospinal fluid acid. From the presence of a high cell count, that is, an average thirty-five cells with a maximum of 110 cells, in the spinal fluid of most of these patients, one may assume a chronic inflammatory condition to have existed; and yet no alteration in reaction was demonstrable. This, however, does not preclude the possibility that acute inflammatory conditions may be associated with a more acid reaction, that is, with an increase in the hydrogen ion concentration. So far as we are aware no positive proof has been brought forth to show that acid substances may form in acute inflammatory conditions of the meninges, and that these may produce an acidulation of the cerebrospinal fluid.

The possibility that the reaction of the fluid may be rendered more acid in cases of infection suggests a therapeutic problem of considerable importance. As has been shown by Crowe¹⁹ hexamethylamin is one of the few antiseptic drugs which can traverse the barrier of the choroid plexuses and pass from the blood stream into the cerebrospinal fluid. But in order to be effective in antagonizing infection, hexamethylenamin must, according to more recent studies,²⁰ be broken up into formaldehyde. This chemical change can occur only in an acid medium. Unless, therefore, it can be demonstrated that the spinal fluid becomes acidulated in the presence of infecting organisms and that hexamethylenamin is actually broken up into formaldehyde, considerable doubt must be entertained concerning the bactericidal power of this drug in combating infections of the meninges.

18. Wingrave, W.: *Jour. Laryngol., Rhinol. and Otol.*, 1915, xxx, 270.

19. Crowe, S. J.: *Bull. Johns Hopkins Hosp.*, 1909, xx, 102.

20. Burnam, C. F.: *THE ARCHIVES INT. MED.*, 1912, x, 324. Hinman, F.: *Jour. Am. Med. Assn.*, 1913, lxi, 1601.

TABLE 4.—THE HYDROGEN ION CONCENTRATION OF THE CEREBROSPINAL FLUID IN SYPHILITIC AFFECTIONS OF THE NERVOUS SYSTEM
[INCLUDING TABLES, GENERAL PARALYSIS AND CEREBROSPINAL SYPHILIS]

Number of Case	Date	Name	Age	Diagnosis	Hospital Number	Wassermann	Cell Count	Name	Noguchi	Reaction of Cerebrospinal Fluid, Value of pH	Reaction of Spinal Fluid, Value of pH	Reaction of Blood, Value of pH
34	8/24/15	H. V.	39	Tuberculous...	10833	B. + + S. F. + +	24	St.	+	8.3	8.15	...
35	8/28/15	L. K.	46	T. b. paresis	10933	B. S. F.	1	0	0	8.3	8.25	...
36	10/12/15	P. A. R.	37	Tuberculous...	Hause 10935	B. + S. F.	0	0	0	8.1	8.0	...
37	8/24/15	N. L.	35	Cerebrospinal syphilis...	307	B. + + S. F. + +	47			7.95 7.95	7.75 7.75	...
38	8/25/15	J. J.	30	Cerebrospinal syphilis...	10941	B. S. F. +	60			8.3	8.15	...
39	8/26/15	J. B.	46	Cerebrospinal syphilis...	10711	B. + + S. F. +	110			8.25	8.15	...
40	8/31/15	S. T.	52	Cerebrospinal syphilis...	17067	B. + + S. F. +	2	0	0	8.2	8.1	7.5
41	10/5/15	D. C.	33	Myelitis hebra.	11164	B. S. F.	3	0	0	8.35	8.2	...
42	10/7/15	H. W.	36	Cerebrospinal syphilis...	11000	B. S. F. +	18	0	0	8.15	8.15	7.75
43	10/12/15	G. F.	42	Cerebrospinal syphilis...	17885	B. + S. F. +	57	—		7.95	7.75	7.65
44	10/19/15	W. S.	47	Cerebrospinal syphilis...	18110	B. + S. F. +	51			...	8.1	...
45	11/16/15	R. Y.	22	Cerebrospinal syphilis...	18641	B. S. F.	22		—	8.1	8.35	...
46	12/8/15	C. A.	36	Cerebrospinal syphilis...	16415	B. S. F. +	5		—	8.4	8.15	7.75
47	12/18/15	S. J.	39	Cerebrospinal syphilis...	10671	B. + S. F. +	5	0	0	8.3	7.95	7.60
Averages	33	8.2	8.0	7.62

* B. means blood; S. F. means spinal fluid.

SUMMARY

The colorimetric method of determining the hydrogen ion concentration of the cerebrospinal fluid gives constant and reliable results. For the reasons given, the method, when applied to cerebrospinal fluid, possesses greater accuracy than in the case of other biologic fluids, notably blood. The simplicity of the technic makes it applicable as a routine procedure in the examination of spinal fluids.

As determined colorimetrically, normal cerebrospinal fluid is more alkaline than blood, the difference in the hydrogen ion concentration of the dialysates of the two fluids being equal to 0.45, the value of pH for cerebrospinal fluid being 8.11; value of pH for blood, 7.66. No alteration from the normal reaction has been noted either in the blood or in the fluid of patients suffering from primary or secondary syphilis or from syphilitic affections of the nervous system. Thus far no study has been made of the reaction of the cerebrospinal fluid in acute inflammatory conditions of the meninges. The demonstration that a change in reaction does or does not occur would have an important bearing upon the value of hexamethylenamin as a therapeutic agent in the prophylaxis and treatment of meningeal infections.

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THE HEXONE BASES OF MALIGNANT TUMORS.

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Interest in the hexone bases of malignant tumors has been stimulated by the recent work of Osborne and Mendel¹ who found a physiological relationship between certain members of this group and normal growth. In prolonged experiments with white rats these investigators showed that, if lysine was omitted from the diet of a growing animal, growth halted. If lysine was added to the diet, the animals began to grow again. On the other hand, in the case of fully grown animals lysine could be omitted from the diet without disturbing the nutritive balance. Thus they distinguished in the diet the factor of growth from that of maintenance. *Lysine is purely a growth-factor.* Experiments with arginine indicated that it had a similar function but as yet this has not been established experimentally.

No satisfactory explanation for the dependence of growth upon either of these amino-acids has been advanced. It seems obvious, however, that the body cannot synthesize these substances and must depend upon outside sources for them. Furthermore, there is a difference between a growing tissue and a fully developed one in its requirement for a given amino-acid, for the adult tissue contains some of this amino-acid; and the difference in requirement would seem to be quantitative.

It is also instructive that certain types of germinating tissue are rich in hexone bases. Spermatozoa are made up largely of a protamine or a histone, combined with nucleic acid. On analysis protamine yields large amounts of diamino-acids, particularly arginine which may constitute as much as 80 per cent of the

¹ Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1912, xii, 473; 1912-13, xiii, 233; 1914, xvii, 325.

protein molecule. In germinating seeds, also, arginine and lysine occur in abundance, and even free. These facts have led me to examine the quantitative relationship between these amino-acids in rapidly growing tissue, namely, human malignant tumors. Of pertinent interest is the work of Miss Slye² who showed in the case of a mouse tumor that heredity is an important factor. In other words the problem of cancer she believes is a problem of growth rather than of infection.

Chemical analyses of tumor tissue are recorded but they are more or less incomplete. Up to the present, strange to say, no one has made a quantitative determination of the hexone bases in tumors. This is especially remarkable since we have reliable quantitative methods for estimating these amino-acids.

Neuberg,³ using the ester method of Emil Fischer in estimating certain mono-amino-acids, concluded that these were present in about the same proportion in cancer as in normal tissue. Bergell and Dörpinghaus⁴ using the same method in the study of carcinoma and sarcoma considered that the proportions of alanine, glutamic acid, aspartic acid, and phenylalanine, were higher than in normal tissues; but the author of this method never claimed that it gave quantitative results.

Selecting a group of three amino-acids, *i.e.*, glycocoll, tyrosine, and glutamic acid, Abderhalden⁵ concluded that the percentage of these in malignant tumors does not vary from normal. Bergell⁶ using the N-distribution method of Hausmann, determined the total N of hexone bases in rat tumors, and concluded that this was much increased. However, this method is grossly inaccurate and conclusions cannot be drawn from such results.

EXPERIMENTAL DATA.

Material for this investigation was obtained through the courtesy of Drs. Rusk and Cooke of the Pathological Department

² Slye, M., *Jour. Med. Research*, 1915, xxxii, 159.

³ Neuberg, C., *Arb. a. d. Path. Inst. z. Berlin*, 1906, 593.

⁴ Bergell, P., and Dörpinghaus, T., *Deutsch. med. Wchnschr.*, 1905, xxxi, 1426.

⁵ Abderhalden, E., and Kautzsch, K., *Ztschr. f. physiol. Chem.*, 1910, lxvi, 69.

⁶ Bergell, P., *Ztschr. f. Krebsforsch.*, 1907, vi, 204.

of the University of California. The specimens were obtained from four to twelve hours after death. Immediately the material was dissected free from all extraneous tissue, weighed, examined, and prepared for analysis. The mass was ground fine through a meat chopper and treated repeatedly with hot water to remove non-protein nitrogen. The water extract was concentrated *in vacuo* to a small volume and the protein in solution precipitated by the addition of several volumes of alcohol. This precipitate was added to the main bulk of protein which had been thrown down by heat. The united material was placed on the water bath, heated to dryness, ground to powder, and extracted repeatedly, in the cold, first with alcohol and later with ether. Then the dried material was weighed and found to represent from 16 to 18 per cent of the original weight. These values are shown in Table I. The dry powder was used for analysis.

Five cases were analyzed, as follows: (1) carcinoma of the larynx; (2) metastatic mass in the liver from a primary carcinoma of the stomach; (3) lymphosarcoma; (4) endothelioma; and (5) a liver metastasis from a primary carcinoma of the ileum. Notes bearing on the histories of the cases, as well as the gross anatomical and microscopic diagnoses, are given below.

TABLE I.

No.	Diagnosis	Weight of fresh material.	Weight of dry substance.	Percentage of dry substance.
		gms.	gm.	
1	Primary carcinoma of larynx	126	21.5	17.0
2	Metastasis to liver from primary carcinoma of stomach	1350	230.5	17.4
3	Lymphosarcoma	228	38.0	16.6
4	Endothelioma. . . .	154	28.0	18.0
5	Liver metastasis from primary carcinoma of ileum. . . .	408	67.0	16.4

Case 1.—Carcinoma of the larynx. Duration of symptoms, 9 months. Anatomical diagnosis: Recurrent carcinoma of the larynx with metastases in regional lymph nodes and invasion of the pharynx, palate, and esophagus. The growth in the neck on section was pale, showing areas of necrosis

and softening alternating with grey and cellular tissue. The regional lymph nodes showed replacement of the greater part of their substance by tumor tissue having a tendency to early central degeneration and softening.

Microscopic examination: Tumor is made up of branching strands of atypical cells evidently of epithelial origin. There are no typical "pearls." Mitotic figures and giant cells are common. Average sections consist of about three-fifths well preserved tumor cells and two-fifths loose edematous stroma containing various wandering cells.

Case 2.—Primary carcinoma of the stomach, with metastasis to the liver. Duration, 4 months. Anatomical diagnosis: Metastases were found in the liver, mesentery, omentum, diaphragm, and right lung. The liver, weight, 3950 gm., was almost all replaced by greyish white metastases which varied in size from a few mm. in diameter to 10 to 15 cm. The larger masses showed central broken down, semi-liquid material. The moderate sized ones showed slight necrosis. The smaller ones showed no gross evidence of degeneration.

Microscopic examination: The growth consisted of a typical carcinoma simplex with only a slight amount of necrosis in the smaller nodules. Mitotic figures are found but are not especially common. Tumor nodules consist of about two-thirds well preserved tumor cells and one-third stroma and other cells.

Case 3.—Sarcoma of the right kidney. Duration of symptoms, 4 months. Anatomical diagnosis: Metastases found in retro-peritoneal and mediastinal lymph nodes and liver. Lobar pneumonia, acute peritonitis. Right kidney, which was the seat of the tumor, showed in the upper half an extensive tumor growth. On the surface were central isolated and confluent opaque grey areas which are surrounded by grey and rather translucent tissue. The entire upper half of the kidney is infiltrated by a firm grey tumor which has involved the cortex more or less completely and much of the medulla.

On section, the tumor shows a pale grey translucent tissue with numerous larger and smaller opaque greyish yellow necroses. Retro-peritoneal lymph nodes are enlarged and matted together, from 1 to 4 cm. in diameter. On section pale grey areas of necrosis are seen. Lymph nodes along the aorta in the posterior mediastinum show similar metastases.

Microscopic examination: There are irregular areas of degeneration and necrosis around which are seen growing tumor cells. These cells are somewhat elongated and hexagonal, and have a considerable amount of pink-staining protoplasm. Cells appear singly or in small nests and are separated by fibrous stroma. They are invading the surrounding tissue. Occasional mitotic figures are found. The picture suggests a sarcoma rather than a hypernephroma. The analysis was made from gland metastases and such glands show about two-thirds of the section to be well preserved large plump cells. The other third consists of lymph strands and stroma.

Case 4.—Endothelioma of the kidney. Anatomical diagnosis: Specimen consists of kidney about four times the normal volume removed at

operation. Superficially it is for the most part irregularly lobulated with low projections held beneath the tense capsule. The surface is pale and smooth except at one pole where there is a nodular mass covering an area about 3 x 5 cm. as if the tumor was projecting through the capsule.

These nodules vary from ochre yellow to brown with spots suggesting hemorrhage. From the pelvis a nodular cream-colored mass projects. On section of the mass the tumor tissue appears as a uniform soft white somewhat edematous growth. There are few vessels and connective tissue septa. The invaded kidney is in great part replaced by tumor tissue except a pale marginal zone of cortex in which it is almost impossible to make out the markings.

Microscopic examination: Shows the growth to be composed of large cells arranged in columns and circular masses with a delicate stroma for the most part, but also with broad tense connective tissue bands in places. In the stroma are thin-walled capillaries. The tumor cells show small rather deeply stained nuclei, a large vacuolated inner protoplasmic zone, and a thin line of cytoplasm at the periphery. Sections stained with Scharlach R show the presence of large amounts of fat. Tumor sections show approximately four-fifths well preserved tumor cells. The rest is made up of stroma and other cells.

Case 5.—Primary adeno-carcinoma of the ileum with metastasis to the liver. Duration of symptoms, 3 weeks. Anatomical diagnosis: Adeno-carcinoma of the ileum with perforation. Metastatic growths in the liver along the bronchial, mesentery, and retro-peritoneal lymph nodes. Primary tumor was situated in the lower ileum. Its center had become necrotic and the bowel wall had perforated, leading to general peritonitis. The liver from which the material was taken weighed 3140 gm. and showed numerous pearl-grey metastatic nodules from 0.3 to 4 cm. in diameter. The larger nodules showed considerable central necrosis.

Microscopic examination: The picture is typical of a very rapidly growing undifferentiated cancer. The glandular arrangement is not in evidence in the metastatic nodules. Stroma is inconspicuous and the tumor cells make up about nine-tenths of the bulk of the nodules in the liver. The amount of hyaline necrosis in the tumor nodules cannot be estimated but the nuclear shadows can still be made out and there is little evidence of autolysis.

Determination of the Hexone Bases.

The method of Kossel and Kutscher⁷ for the determination of the hexone bases has been modified by Osborne, Leavenworth,

⁷ Kossel, A., and Kutscher, F., *Ztschr. f. physiol. Chem.*, 1900 01, xxxi, 165. Kossel, A., and Pringle, H., *ibid.*, 1906, xlix, 318.

and Brautlecht,⁸ also by Steudel,⁹ and by Van Slyke.¹⁰ In this work all these modifications were employed and the method followed was essentially that used by Van Slyke in his analysis of casein. The material was hydrolyzed with ten volumes of 20 per cent hydrochloric acid for a period of thirty-two to thirty-eight hours. The progress of the hydrolysis was followed until complete by determinations of the amino nitrogen with the Van Slyke apparatus. Lysine was isolated as the picrate, weighed, and its purity controlled by analysis of the amino nitrogen. Arginine and histidine were isolated as picrolonates according to the method of Steudel, and purity was controlled by melting point and analysis of the nitrogen. In all cases the values for the bases were calculated from the salts and not from the determination of the nitrogen in solution. The analytical data are given in Table II.

The results shown in the table indicate that in the malignant tumors studied the amount of hexone bases was fairly uniform. The arginine N expressed in per cent of the total nitrogen was between 11.63 and 13.65, an average of 12.42 per cent. The highest figures found, 12.8 and 13.65, were in Cases 2 and 5. These were liver metastases and consisted almost exclusively of carcinoma cells. In the other cases beside the tumor cells more or less connective tissue stroma was present. In Cases 3 and 4 approximately a third to a fifth of the tumor mass consisted of connective tissue.

The histidine N represents 3.88 to 5.58 per cent or an average of 4.86 per cent of the total N. The lysine N represents 9.89 to 12.65 per cent, an average of 11.23 per cent of the total N. It is noteworthy that if one of the members of this group is increased, the other two also are relatively more abundant. Perhaps these three substances are bound in some way as a unit in the protein molecule. The work of Wakeman¹¹ also favors this view.

The interpretation of the results of these analyses is facilitated by reference to similar analyses of normal tissues. Unfortu-

⁸ Osborne, T. B., Leavenworth, C. S., and Brautlecht, C. A., *Am. Jour. Physiol.*, 1908 09, xxiii, 183.

⁹ Steudel, H., *Ztschr. f. physiol. Chem.*, 1902-03, xxxvii, 219; 1905, xlv, 157.

¹⁰ Van Slyke, D. D., *Jour. Biol. Chem.*, 1913-14, xvi, 535.

¹¹ Wakeman, A. J., *ibid.*, 1908, iv, 119.

TABLE II.

No.	Diagnosis	Wet weight of substance analyzed, gm.	Length of hydrolysis, hrs.	Total N in hydrolyzed tissue, gm.	Arginine per cent, gm.	Histidine per cent, gm.	Nitrogen of the bases,				N of bases in percentage of total N,			
							Arginine	Histidine	Lysine	Total base	Arginine	Histidine	Lysine	Total base
							gm.	gm.	gm.	gm.	percent	percent	percent	percent
1	Carcinoma of larynx	20	34	2.1355	2.0195	0.8270	0.2581	0.0829	0.2192	0.5602	12.06	3.88	10.25	26.20
2	Liver metastasis from primary carcinoma of stomach	40	33	4.4906	4.4980	2.4743	0.5750	0.2480	0.5682	1.3812	12.80	5.52	12.65	30.75
3	Lymphoma sarcoma	36	38	4.1250	3.8562	1.9918	0.4930	0.1996	0.4675	1.1601	11.95	4.84	11.32	28.14
4	Endothelioma	28	32	3.0652	2.7881	3.5362	0.3565	0.1367	0.3030	0.7962	11.63	4.46	9.89	25.94
5	Liver metastasis from primary carcinoma of ileum	40	35	5.595	5.9695	3.1190	0.7642	0.3125	0.6752	1.7519	13.65	5.58	12.06	31.32
Average											12.42	4.86	11.23	28.47

nately, however, few are at hand. A series of analyses of various organs including those of man was made by Wakeman who employed essentially the methods I have used. He found that the percentages of the hexone bases for a given tissue vary but slightly in different animals and not greatly in different organs of the same animal. For comparison with my results I reproduce in Table III the figures obtained by Wakeman.

In Table IV are given the percentages of the hexone bases in a few isolated proteins.

TABLE III.

Animal.	Organ.	Arginine N in percent- age of total N.	Histidine N in percent- age of total N.	Lysine N in percent- age of total N.	Total base N in per- centage.
*Adult man..	Normal liver	6.88	2.01	6.60	15.48
*Adult man..	Normal liver	5.61	1.76	5.54	12.91
*Dog	Normal liver	8.32	2.31	4.81	15.44
*Horse.....	Normal liver	5.72	1.76	5.64	13.12
*Dog.....	Kidney	4.19	2.51	6.05	12.75
**Dog.....	Placenta at full term	4.3	0.30	3.5	8.1
**Ox.....	Muscle	7.5	1.8	7.6	16.9
Average for somatic tissue		6.07	1.78	5.68	13.52
Average for tumor		12.42	4.86	11.23	28.48

* Wakeman.

** Koelker and Slemons.

TABLE IV *

Protein.	Arginine.	Histidine.	Lysine.	Total base N.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
** Egg albumin ..	10.2	2.7	4.3	17.2
** Gliadin (wheat)....	6.7	2.4	?	9.1
** Casein.	8.0	4.0	8.6	20.6
** Lactalbumin (milk)	6.2	2.4	9.2	17.8

* These figures for nitrogen percentages are calculated from the amino-acid percentages by factors given by Van Slyke (*Ber. d. deutsch. chem. Gesellsch.*, 1911, xlv, 1690).

** Osborne and Mendel.

A comparison of the amounts of hexone bases in malignant tumors with the amounts in various normal adult tissues shows an increase in the former. Generally, the yields of arginine, lysine, and histidine obtained on hydrolysis of malignant tumors are over 100 per cent greater than those obtained on hydrolysis of normal somatic tissue. The quantities of these bases are also greater than those found in any isolated protein except the protamines and histones.

Attempts to isolate or to obtain qualitative tests for histone and protamine, using the fresh tumor tissues, were unsuccessful.

These results in conjunction with those of Osborne and Mendel may explain the action of lysine in promoting normal growth on the ground that this diamino-acid which cannot be synthesized in the body is an inherent requirement of the newly forming protein and is embodied in the structures of the latter molecule. If this is true for lysine, it seems probable that further experiments with arginine and histidine will show these bases to have a similar rôle.

It is pertinent that Sweet¹² and Rous¹³ have recently demonstrated that when white rats having carcinoma are fed on a diet deficient in the hexone bases the tendency to metastasize is diminished. In some of their cases, however, the tumor grew as rapidly as ever, and in that event there was a correspondingly rapid emaciation of the body. This fact, it seems to me, may indicate that the somatic tissue was broken down to furnish these substances to the rapidly growing tumor cells.

Whether such high percentages of the hexone bases as we have found is a peculiarity of malignant tumors, or whether such an increase will be found in normal rapidly growing tissue is a problem now under investigation in this laboratory.

SUMMARY.

In five cases of malignant tumor the quantity of hexone bases was approximately double that found in normal tissue.

These results suggest a new approach to the study of the cancer problem, especially as it relates to metabolism.

¹² Sweet, J. E., Corson-White, E. P., and Saxon, G. J., *ibid.*, 1913, xv, 181.

¹³ Rous, P., *Bull. Johns Hopkins Hosp.*, 1915, xxvi, 146.

THE MECHANISM OF THE SPARING ACTION OF CARBOHYDRATES ON PROTEIN METABOLISM.

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Voit,¹ Rubner,² Benedict,³ Sivéén,⁴ Landergren,⁵ and others have repeatedly demonstrated that ingested carbohydrates exert a sparing effect on the metabolism of body protein. This sparing action is considerably more marked with carbohydrates than with fats. It is possible with the ingestion of large amounts of carbohydrates to reduce the nitrogen output in man from the starvation figures of 8 or 10 gm. to as low as 3 gm. If the carbohydrates are omitted from the diet or even replaced by fat, the nitrogen output again increases to more than double this minimum. Loewi,⁶ Luthje,⁷ Lesser,⁸ and others further showed that carbohydrates are necessary for the utilization of the split products of protein. Without carbohydrates there is no retention of nitrogen on feeding mixtures of the abiuuret split products. In explanation of this, Luthje thought that an amino sugar was formed. There is, however, no experimental evidence to support this view. According to Landergren, since under physiological conditions fats cannot supply this want, the proteins are broken down to yield the sugar

¹ Voit, C., *Hermann's Handb. Physiologie*, Leipsic, 1881, 140.

² Rubner, M., *Die Gesetze des Energieverbrauchs bei der Ernährung*, Vienna, 1902.

³ Benedict, F. G., *Carnegie Institution of Washington, Publication No. 77*, 1907.

⁴ Sivéén, V. O., *Skand. Arch. Physiol.*, 1900, x, 91; 1901, xi, 308.

⁵ Landergren, E., *Skand. Arch. Physiol.*, 1903, xiv, 112.

⁶ Loewi, O., *Arch. exp. Path. u. Pharm.*, 1902, xlviii, 303.

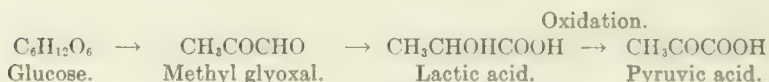
⁷ Luthje, H., *Arch. ges. Physiol.*, 1906, cxiii, 547.

⁸ Lesser, E. J., *Z. Biol.*, 1904, xlv, 497.

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which is lacking. This results in an increase in the nitrogen output. Cathcart⁹ suggested that carbohydrate is essential to protein synthesis, and this view is held also by Janney.¹⁰

Sugar, except when stored as glycogen, is dissociated in a definite way in the body into simpler molecules. An idea of the relationship of glucose to the dissociation products may be gathered from the following scheme:



Of special interest in this connection is the fact demonstrated by Otto Neubauer that keto and hydroxy acids identical with those arising in the course of sugar catabolism may result from the dissociation of proteins in the body. For example, alanine is deaminized to form pyruvic acid, which by reduction gives rise to lactic acid according to the following scheme:



It has been shown by a number of workers that the above reactions are reversible. For example, lactic acid and pyruvic acid may give rise to glucose in a phlorhizinized animal. The increased glycosuria in phlorhizinized animals following ingestion of proteins is a further evidence of the reversal of this reaction. That the second of these reactions is reversible, namely, the synthesis of hydroxy and keto acids into amino-acids, has been demonstrated by Knoop¹¹ as well as by Embden¹² and others. They have shown that on perfusing the isolated liver with lactic or pyruvic acid, nitrogen can be added on and alanine formed. If this process of retaining nitrogen by dissociation products of sugar to form new amino-acids, and hence proteins, occurs on a large scale in the body, it will explain why ingestion of carbohydrates

⁹ Cathcart, E. P., *The Physiology of Protein Metabolism*, London, 1912, 121.

¹⁰ Janney, N. W., *J. Biol. Chem.*, 1916, xxiv, p. xxx.

¹¹ Knoop, F., *Z. physiol. Chem.*, 1910, lxxvii, 439.

¹² Embden, G., and Schmitz, E., *Biochem. Z.*, 1910, xxix, 423.

spares body protein. To test this point, the sparing effect on the nitrogen output of ingesting lactic and pyruvic acids as compared with the sparing action of equivalent amounts of undissociated carbohydrates was undertaken in these experiments.

EXPERIMENTAL.

Large healthy dogs were used for the experiments. They were allowed to fast for 5 or 6 days. When the nitrogen output became constant, lactic acid, pyruvic acid, or cane sugar was given by stomach tube. The lactic acid was partly neutralized by calcium hydroxide; pyruvic acid was neutralized with sodium hydroxide. For comparative purposes the sparing action of cane sugar was first determined for each dog. The urine was collected over 24 hours by catheterization. During fasting the dogs received daily a liter of water, and the carbohydrates and acids were administered in an equivalent dilution, so that the daily quantity of urine was kept constant. The results of the experiments are tabulated in Tables I to III.

TABLE I.

Experiment 1.—A healthy bitch weighing 19.5 kg. Cane sugar was given on the 7th day; lactic acid partly neutralized with calcium hydroxide was given by stomach tube on the 10th day.

Day.	Food.	N in the urine. <i>gm.</i>	Remarks.
5	Fasting.....	2.86	3.32 gm. of <i>l</i> -lactic acid were recovered in the urine.
6	".....	2.92	
7	95 gm. cane sugar.....	1.66	
8	Fasting.....	2.56	
9	".....	2.84	
10	100 gm. lactic acid.....	1.82	
11	Fasting.....	2.48	
12	".....	3.03	

TABLE II.

The Effect of Lactic Acid on the N Output of the Dog.

Experiment 2.—Dog weighing 18.6 kg. In this experiment doses of cane sugar and lactic acid were given over 3 days in amounts of 80 and 80 gm. respectively.

Day.	Food.	N in the urine. gm.	Remarks.
5	Fasting	3.35	
6	"	3.49	
7	76 gm. cane sugar..	2.25	
8	76 " " "	1.74	
9	76 " " "	1.27	
10	Fasting	1.44	
11	"	2.22	
12	"	3.19	
13	"	3.40	
14	80 gm. lactic acid ..	2.68	Traces of lactic acid found in the urine.
15	80 " " "	2.19	" " " " " " " "
16	80 " " "	1.65	" " " " " " " "
17	Fasting	2.03	
18	"	2.55	

TABLE III.

Experiment 3.—Same dog as in Experiment 2. Pyruvic acid neutralized with sodium hydroxide was given in doses of 80 gm. per day for 3 days. The pyruvic acid was prepared in the laboratory by the distillation of tartaric acid and potassium hydrogen sulfate. It was freshly distilled before use.

Day.	Food.	N in the urine. gm.	Remarks.
6	Fasting	3.52	
7	"	3.41	
8	80 gm. pyruvic acid.	2.88	
9	80 " " "	2.44	
10	80 " " "	2.18	Traces of <i>l</i> -lactic acid were detected in the urine.
11	Fasting	2.56	
12	"	2.85	
13	"	3.36	

Following the ingestion of cane sugar there was the usual reduction of the nitrogen output. In these experiments this reduction was down to approximately 53 per cent of the fasting nitrogen when 100 gm. of carbohydrates were given on 1 day only; following the administration of 76 gm. a day for 3 consecutive days, the nitrogen output was reduced to about 37 per cent of the fasting nitrogen. These figures serve as a basis of comparison for the sparing action of equivalent amounts of lactic and pyruvic acids. In Experiment 1 where 100 gm. of lactic acid were given, the nitrogen output was reduced from 2.84 to 1.82 gm., or 64 per cent of the fasting nitrogen. 3.32 gm. of *l*-lactic acid were recovered from the urine. It is apparent that not quite all of the lactic acid had been utilized, but the reduction in the nitrogen output to 64 per cent as compared with 53 per cent for an equivalent amount of cane sugar indicates that lactic acid spares protein to practically the same extent as do carbohydrates. In Experiment 2 lactic acid was given in amounts of 80 gm. on 3 consecutive days. The daily nitrogen output was reduced from 3.4 gm. to 1.65 gm. on the 3rd day, or 48 per cent of the fasting nitrogen as compared with a reduction to 37 per cent following equivalent amounts of cane sugar. There were traces of lactic acid found in the urine, so that it is likely that had all the lactic acid been utilized reduction in nitrogen output would have been fully as great as that following carbohydrates. In Experiment 3 the effect of ingesting pyruvic acid was studied. 80 gm. doses per day were given for 3 days. The nitrogen output fell from 3.41 to 2.18 gm. on the 3rd day, or 64 per cent as compared with 37 per cent following carbohydrates, and 48 per cent following lactic acid. There were no toxic symptoms apparent following administration of these amounts of pyruvic acid in the form of the sodium salt. A trace of *l*-lactic acid was detected in the urine on the 3rd day following the administration.

DISCUSSION OF RESULTS.

Lactic acid exerts practically the same sparing action on protein metabolism as carbohydrates. Following pyruvic acid the sparing action is very distinct but less marked than that following lactic acid and carbohydrates. The dissociation of glucose in

the body is by way of lactic acid as one of the chief intermediate steps. Lactic acid can be oxidized in the body to pyruvic acid. As pointed out in the introductory remarks, it has been shown that lactic acid as well as pyruvic acid can add on ammonia nitrogen to form alanine. When this process is operative, nitrogen arising from catabolism of body proteins instead of being excreted is utilized to synthesize new protein. It is true that the above reactions are reversible, as has been shown in phlorhizinized animals; but the normal catabolism of glucose is in the direction indicated, giving rise to simpler molecules, which in turn may be in part combined with nitrogen to synthesize protein. That this fixing of catabolized nitrogen by the dissociation products of glucose to form new proteins is the true mechanism of the sparing effect of feeding carbohydrates on the nitrogen output gains further support in the experiments here reported.

STUDIES ON THE FORMATION AND
ANTIGENIC PROPERTIES OF
CERTAIN COMPOUND
PROTEINS*

BY

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INTRODUCTION

Despite the large amount of work of many investigators, the nature of biological specificity remains obscure. It is well known that if an animal be immunized with the serum from an animal of another species, antibodies are formed in the serum of the treated animal which will react specifically with the serum from an animal of the same species as that from which the serum used in the injection was taken. Instead of serum, washed corpuscles, extracts of body tissues, milk, egg-white, bacteria, pure proteins, etc., may be used to produce an immunity, and the presence of proper amounts of antibodies (antitoxins, agglutinins, precipitins, fixing antibodies, etc.) can be demonstrated by the appropriate reaction. Antibodies, in general, can be produced in animals only by protein substances and not by carbohydrates and fats. Some evidence has been brought forward to show that lipoids¹ may be antigenic, but the recent work of Fitzgerald and Leathes² and Thiele and Embleton³ shows that if the lipid be pure it is non-antigenic, and the apparent antigenic properties reported by various workers at times may have been due to admixed proteins. There

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is also some evidence brought forth to show that glucosides⁴ are antigenic but doubt⁵ has been cast on this work, since it is open to the same objection.

Instead of establishing an immunity to a foreign serum or protein substance, an animal may be made more susceptible, so that on giving it a subsequent injection of the homologous substance, it will suffer an anaphylactic shock. This very interesting phenomenon has been studied at length by Portier and Richet,⁶ Arthus,⁷ Otto,⁸ Rosenau and Anderson,⁹ Richet,¹⁰ Von Pirquet and Shick,¹¹ Biedl and Kraus,¹² Friedberger and Hartoch,¹³ Gay and Southard,¹⁴ Nicolle,¹⁵ Friedemann,¹⁶ Pearce and Eisenbrey,¹⁷ Auer and Lewis,¹⁸ Jobling,^{18a} and many others. The subject is reviewed at length by Richet,¹⁹ Gay,²⁰ Rosenau and Anderson,²¹ Doerr,²² and Zinsser.^{22a}

In general, the biological reactions appear to be specific, though probably only relatively so. In this respect the precipitin reaction²³ has been studied at length. Thus Myers²⁴ and Uhlenhuth²⁵ noted that rabbits immunized with egg-albumin gave precipitins which acted not only with the substance derived from one species but also with egg-albumin of other species, the reaction being more marked with the homologous than with the heterologous substance. The experiments were further extended by Nuttall²⁶ and Graham-Smith,²⁷ who likewise obtained precipitin reactions with heterologous egg-albumins. Welsh and Chapman,²⁸ by saturation experiments, found in avian egg-white antiserum the presence of a general avian antisubstance together with the specific antisubstance. Observations indicative of the presence of a general, as well as a specific antibody, in the precipitin reaction were also made by Levene²⁹ and by Umber.³⁰ On the specificity of serum precipitins, observations have been made by Uhlenhuth,³¹ Wassermann and Schütze,³² Ewing and Strauss,³³ Ewing,³⁴ Stern,³⁵ Wassermann,³⁶ Grünbaum,³⁷ Strube,³⁸ Linossier and Lemoine,³⁹ and Gay.⁴⁰ In general, the specificity of the precipitin reaction is shown, though it is quite apparent that precipitins showing group reactions for closely related biological substances can be obtained.

The anaphylactic reaction appears to be no less markedly specific than the precipitin reaction, as shown by the recorded

work on the subject. Thus Rosenau and Anderson⁴¹ found that guinea-pigs sensitized with a foreign serum reacted more violently when subsequently given an injection of serum from an animal of the same species than when injected with the serum from an animal of another species. However, a guinea-pig sensitized to horse serum did not react to a subsequent injection of egg-white or milk. They were also able to demonstrate that a guinea-pig could be sensitized with three different substances, viz., egg-white, milk, and horse serum and subsequently react on a second injection to each of these substances. The use of widely differing protein substances eliminated any group reaction. Later they found that reactions could be obtained between substances closely related biologically. Gay and Southard^{14d} cite experiments to show that the anaphylactic sensitization produced in guinea-pigs by the previous injection of horse serum, egg-white or milk is only relatively specific, depending on whether any partial intoxication by a heterologous substance has been produced. Thomsen⁴² in accordance with the work of Rosenau and Anderson found that an animal sensitized to a foreign serum reacted anaphylactically only with a homologous serum and not to any foreign sera unless the serum was from an animal showing close biological relationship. The reports of various observers seem to indicate that lens protein showed no specificity, but Kapsenberg⁴³ found that it possesses a slight but distinct specificity when tested by the anaphylaxis reaction. The experiments of Wells,⁴⁴ Besredka,⁴⁵ Uhlenhuth and Haendel,⁴⁶ Kraus,⁴⁷ Andrejew,⁴⁸ Pfeiffer and Mita,⁴⁹ Friedberger and Goretti,⁵⁰ Krusius⁵¹ and others show that the anaphylactic reaction is specific, though a lesser reaction may be obtained with a closely related substance which may be biologically different.

With the alexin fixation reaction,⁵² results essentially in accordance with those previously cited for the precipitin and anaphylactic reactions have been obtained, showing that cross-fixation may be obtained with protein substances closely related chemically or biologically. Thus Gengou⁵³ states that by injecting rabbits with large doses of cow's milk, egg-white, horse fibrinogen, or heated dog serum, sensitizers are formed analogous to those described by Bordet in bacteriolytic and hemolytic sera

and the sensitizers in these immune sera may react with substances other than those used for immunization. Thus the serum of an animal injected with hen blood reacts with hen egg-white. Ballner,⁵⁴ Dunbar,⁵⁵ Wendelstadt and Fellner,⁵⁶ working with vegetable proteid substances, found that the reactions were specific. Bauer⁵⁷ finds that the alexin-fixation reaction is more specific than the precipitin and is able to differentiate different protein substances from the same species. Brück⁵⁸ also finds that the alexin-fixation reaction is very specific, though Fitzgerald⁵⁹ does not agree with his findings that racial differences can be determined by this reaction.

Thus far we have considered only the specificity of sera, tissue extracts and closely related biological substances, all of which are of indefinite chemical composition, being either mixtures of a number of different proteins or a complex chemical combination of loosely bound proteins such as Hardy⁶⁰ considers exists in blood sera. To interpret the large amount of accumulated data on specificity of body tissues and fluids from the standpoint of chemical constitution is impossible. We can merely suspect that possibly the interreaction between two protein substances such as egg-white derived from different species is due to the presence of proteins varying but little in their composition, or to the presence of common groups within certain protein molecules in the substance, or to the linkage of certain proteins not wholly in common, which, when combined, would make the combination react not only with itself but individually with the several proteins forming the combination. It is clear that real progress can be made from a chemical standpoint only by working with pure proteins or combinations of pure proteins whose composition is known.

The necessity of working with pure proteins was realized some time ago. Gengou⁶¹ in his fixation experiments used casein, that protein being easily obtained in a comparatively pure state. Fleischer⁶¹ was able to obtain precipitin reactions with purified casein from different species. Wells,⁶² working with recrystallized egg-albumin, found that it was much more active antigenically than the unpurified egg-white from which it was derived. He noted that the toxicity of proteins which were

coagulated by alcohol or by heating was destroyed or reduced while other proteins which were not coagulated were unaffected by heat. His experiments yielded the interesting information that gelatine was unable to sensitize or intoxicate; that complete tryptic digestion of a protein causes it to lose its toxic properties for animals previously sensitized to that protein, and that there was a difference in the toxicity of zein and gliadin. Continuing this work, Wells⁶³ later found that ovovitellin from hens' eggs is entirely different, when tested anaphylactically, from ovomucoid or crystallized egg-albumin. This demonstrated the presence of a number of different antigens in egg-white. He confirmed the previous work of Fleischer⁶¹ that casein from various species interreacts, showing that chemically the casein from different species is identical. Wells further carried out experiments with histones, nucleic acid and nucleoproteins, his results showing that not all proteins or protein derivatives are antigenic. In fact, he found that the histone from cod testes was very toxic, confirming Taylor's⁶⁴ previous observation that protamine from salmon sperm was toxic.

Later Wells⁶⁵ working with Osborne, to whom we are indebted largely for our knowledge of the vegetable proteins, carried out the most comprehensive immunologic experiments with pure vegetable proteins that have so far been done. They found that all the proteins studied caused anaphylaxis in sensitized animals, the toxicity differing with the particular protein used. Within certain limits the vegetable proteins were specific, though groups which interreacted were found which established the close relationship of the gliadins from wheat and rye and the legumins of the pea and vetch. Guinea-pigs sensitized with gliadin from wheat or rye gave strong anaphylactic reactions with hordein from barley, but not so strong as with the homologous protein. These experiments indicate that specificity is determined by the chemical constitution of the protein molecule instead of by its biological origin. Since an animal sensitized to a particular protein reacts, though not so strongly, with a protein of different biological origin, though probably closely related chemically, it would appear that the entire protein molecule is not involved in the specificity but only certain groups of which there may be

a number in each molecule. Thus proteins having one or more groups in common would interreact to a greater or less extent.

While the work of Wells and Osborne was in progress, White and Avery⁶⁶ working with pure edestin and gliadin obtained some very interesting results. By means of hemagglutination, the precipitin reaction, alexin fixation, and the anaphylaxis reactions, they were able clearly to differentiate edestin from gliadin. On hydrolyzing edestin with an alcoholic solution of sodium hydrate, fatal intoxication in guinea-pigs similar to anaphylactic shock was produced on intravenous injection.

Elliott,⁶⁷ continuing the work begun by Wells on mucin, found that the glycoproteins studied by him were antigenic, but not so markedly so as simple proteins. The specificity of mucin was marked, though to a lesser degree reactions were obtained with the serum of the homologous animal and with the other mucins almost as well as with the blood serum. The reaction with the other mucins was independent of species and the antiserum did not react with the blood serum of the different species. His work gave support to the theory that the chemical structure of the antigen is the determining factor rather than its biological origin.

Believing that the specificity of a protein is dependent on the structure of the amino-acid groups, Obermeyer and Pick,⁶⁸ by iodizing, nitrifying or diazotizing rabbit serum, found that such altered proteins would produce precipitins which would react only with the same changed proteins without regard to the species from which the protein was derived, but not with the original unchanged protein. Landsteiner and Jablons⁶⁹ treated egg-white with acetic anhydride and obtained antigens which showed very slight species specificity but more pronounced structure specificity. This they attribute to the entrance of the acetyl group into the structure of the protein molecule. Wells⁶² and Pick and Yamanouchi⁷⁰ were unable to obtain the same effect on the specificity of the anaphylactic reaction as found by Obermeyer and Pick with the precipitin reaction. The toxicity of the iodized protein appears to be diminished. Not all protein substances are able to stimulate the production of antibodies or even to sensitize for anaphylactic shock. Some are even primarily toxic. The previously cited work of Wells⁶² with gelatine and

that of Taylor⁶¹ and Wells⁶² with protamine illustrates these two points.⁷¹ Earlier workers⁷² with peptone and partial digestion products of proteins noted a retardation of blood coagulation. Nolf,⁷³ and Underhill⁷⁴ have shown that the proteoses are toxic when injected into animals, and Biedl and Kraus¹² attempted to show that anaphylaxis is produced by substances similar to those causing peptone intoxication, but this view has been criticised by Richet⁷⁵ and Wells⁶² and apparently shown to be incorrect. Zunz⁷⁶ further showed that active and passive anaphylaxis can be produced in both rabbits and guinea-pigs by the three primary albumoses, but not by thioalbumose or the so-called secondary proteoses, or by Siegfried's pepsinfibrinpeptone- β , or by the products of the peptic, tryptic or ereptic digestion of fibrin. Synalbumose was found to sensitize only.

It would appear, then, that a protein can be hydrolyzed to a point where it loses one or more of its biological properties or even becomes non-toxic.⁷⁷ In attempting to correlate the structure of proteins with their toxicity on initial injection, Schittenhelm and Weichardt⁷⁸ found that globin, protamin, and histone were toxic as shown by lowering of the blood pressure, modified respiration, drop in temperature, and in some cases, death. In comparison, the true proteins such as casein produced no such symptoms when injected into animals. These results are in accordance with their general findings that proteins can be rendered non-toxic either by combining with other substances which are not toxic (e.g., nucleohistone) or by splitting them into comparatively simple products which are non-toxic. Bürger⁷⁹ has likewise found that amino-acids, pure albumoses, protamines and acid albumins are not adapted to sensitize in anaphylaxis. Gay and Robertson⁷⁷ carried out experiments with casein and its split products. They found that casein and paranuclein have distinct antigenic properties as shown by the anaphylaxis and alexin-fixation reactions. A solution of the products of the complete peptic digestion of casein was toxic for animals but not more markedly so in animals which had previously been sensitized to paranuclein or itself. This preparation failed to give a fixation reaction with an anti-casein or anti-paranuclein serum. The principal amino-acids of casein, glutamic acid and leucin, mixed in the same proportions

as present in the parent protein showed no antigenic properties, neither were they toxic. By the synthetic action of pepsin by which paranuclein-A (Robertson)⁸⁰ is built up from the products of the complete peptic digestion of casein, Gay and Robertson showed the genesis of an antigenic substance from non-antigenic products. Paranuclein and the synthetic product when tested by reactions of anaphylaxis and alexin fixation with an anti-casein serum proved to be identical.

Most interesting is the work of Vaughan⁸¹ and his collaborators in splitting off different products from the protein molecule in the attempt to attribute to some particular group the property of intoxication and to another the power of sensitization. By treating large masses of bacteria grown in special apparatus with alcoholic potash they were able to split the cellular substance into two fractions, one, the carbohydrate group, insoluble in alkaline alcohol and non-toxic, while the other is soluble in the alcohol and exceedingly toxic for animals, giving symptoms similar to anaphylaxis. The same poison was obtained irrespective of what bacterial substance was used. Extending the work further they were able to obtain this toxic fraction from any pure protein, irrespective of its origin. The toxic fraction split from proteins by alcoholic potash does not sensitize and shows no specificity. Their theory, then, is that all proteins contain two groups, one of which is toxic and physiologically the same in all proteins, to which is attributed the symptoms shown by a sensitized animal on subsequent injection of the protein; the other a sensitizing group which differs in proteins and to which the specificity of the protein can be attributed. They have been able to obtain specific sensitizing groups from certain bacteria and certain pure proteins. Neither the toxic nor the sensitizing fraction has as yet been obtained in a state of chemical purity or its constitution established.

Attacking the problem in a somewhat analogous way, Gay and Adler⁸² by fractional precipitation of blood serum with ammonium sulphate, found that the more ammonium sulphate added the more toxic and less sensitizing the fractions became. The first fraction (euglobulins), obtained by one-third saturation with ammonium sulphate, was found to be as highly sensitiz-

ing as untreated horse serum but non-toxic for sensitized animals. This observation strengthened Gay and Southard's theory that two separate components exist in sera, one of which (anaphylactin) sensitizes and the other of which intoxicates. Following along the same lines as Gay and Adler, Doerr and Russ⁸³ sensitized guinea-pigs with bovine serum and obtained symptoms of anaphylaxis on a subsequent injection of the globulin fraction of the bovine serum. By reversing this procedure identical results were obtained. Repetition of these experiments with horse serum also gave similar results. The albumins were neither sensitizing nor intoxicating provided they were sufficiently purified. From these results Doerr and Russ⁸³ conclude that the sensitizing substance is identical with the substance which exerts a toxic action on reinjection. Braun⁸⁴ likewise found that the anaphylactic reacting substance was precipitated from serum in the globulin fraction.

Hall⁸⁵ found that conglutinin is precipitated from heated (56° C) bovine serum by ammonium sulphate mainly in the euglobulin fraction. By filtering through a Berkefeld filter most of the conglutinin of heated (56° C) bovine serum was retained, but if fresh bovine serum was filtered through a Berkefeld filter and then heated to 56° C the conglutinin was found to have passed through, apparently unaltered. Here we probably have an association of molecules to form complexes of larger size, as shown by the inability to pass through the Berkefeld filter.

Armit⁸⁶ prepared crystallized egg-albumin according to the well-known method of Hopkins in which ammonium sulphate is used as a precipitating reagent, and was unable, on hydrolyzing it with alcoholic potash, to produce a sensitizing and an intoxicating substance such as Vaughan has described. From this Vaughan concludes that the precipitating salt has changed the nature of the protein, and considers it probable that the anaphylactin obtained by Gay and Adler contains the poisonous group but so combined that it is not set free *in vitro* or *in vivo*. For this assumption, however, there is no direct proof.

Thus far we have considered the attempts made by experimenters to account for the various phenomena connected with specificity, immunity and sensitization, nearly all of whom

attacked the problem from the analytical standpoint, i.e., to obtain by means of purification, chemical separation, or from similarity of chemical structure, the individual determining factors to which these phenomena might be attributed. These attempts have met with a certain amount of success. But since our knowledge of the chemical constitution of pure proteins and even of the methods for the separation of pure proteins from complex protein substances is far from complete, this method of attack has fallen short of its goal. The other alternative is offered by the synthesis of proteins or protein compounds which would possess distinctive properties. Although the work of Emil Fischer and his pupils has added greatly to our knowledge of the building of proteins, yet it has not reached the stage where it can be used for the study of such complex problems as biological specificity. Attempts to attack specificity have so far been limited to the synthesis of proteins through the reversible action of ferments or the combinations of two pure protein substances to form a protein complex. This may be done by the combination of two non-antigenic proteins, one or both of which may be toxic or both non-toxic, to form a compound having properties different from those of either of the constituents; or by the combination of an antigenic and a non-antigenic protein, the latter of which may be toxic. It is conceivable that a large protein complex, such as Hardy⁶⁶ assumes exists in blood serum and to which Robertson⁶⁷ has suggested the specificity of animal tissues and fluids might be due, could be built from pure, known proteins. Since there are considerable differences in the content of various proteins in the blood sera of different species of animals as shown by the recent work of Robertson and collaborators,⁶⁸ the protein complexes would also differ, and this might account for the differences in the crystalline form of hemoglobin observed by Reichert and Brown⁶⁹ in different species, the form of the crystal being determined by the protein complexes in the serum.

Reference has already been made to the work of Schittenhelm and Weichardt,⁷⁰ showing that when a toxic histone is combined with nucleic acid, which is apparently non-antigenic, in the form of nucleo-histone it becomes non-toxic. Toxic globin when combined with hematin as hemoglobin is likewise non-toxic. Nucleo-

proteins of animal origin have been claimed by Beebe⁸⁸ and by Guerrini⁹⁰ to show organ specificity, but this has been seriously questioned by Pearce⁹¹ and a number of other workers.

Gay and Robertson⁹² prepared two compound proteins, globin caseinate and protamine caseinate, both being combinations of an antigenic non-toxic protein with a non-antigenic toxic protein. Their experiments yielded very interesting results. Toxic globin when combined with casein gave a compound, globin caseinate, which gave antibodies when tested by the alexin-fixation reaction, which reacted not only with itself but with casein and globin individually. By this combination globin was rendered antigenic. Shaking an antiglobin caseinate serum with globin and subsequently filtering removed the antibodies for globin, so that the filtered serum reacted only partially with the compound protein, not at all with globin but completely with casein. Animals sensitized with globin and subsequently injected with globin caseinate showed no symptoms; sensitization with globin caseinate gave marked symptoms on reinjection of the compound protein; animals sensitized to the compound protein reacted anaphylactically when subsequently injected with casein; a single injection of globin caseinate did not produce symptoms in animals. Though the combination of globin with casein rendered globin antigenic when tested by the alexin-fixation reaction, it did not show sensitizing action as tested by a subsequent injection of the compound protein. Combination of protamine with casein did not render protamine antigenic, though the antiserum to protamine caseinate showed antibodies to casein and to itself as tested by the fixation reaction. The compound, however, was non-toxic. In this case the antigenic properties of the compound are entirely due to casein. From these experiments it is quite conceivable that a protein complex may be built up from non-antigenic (also toxic) components which would itself be antigenic and non-toxic but would produce an antiserum which would react to certain or all of the components used to build up that complex. This might determine specificity.

The present work is a continuation of the work on the study of compound proteins begun by Gay and Robertson some time ago. Before much work was done in preparing new compounds

it seemed of vital importance to study the mechanism of the reaction which takes place between proteins to form a compound, inasmuch as no absolute criteria were at hand to determine such compound formation. The work with which this paper therefore is concerned is divided into two parts—(1) a study of the reactions which take place when two proteins unite to form a compound; (2) a study of the antigenic properties of several proteins compounded in this manner.

I

CHANGES IN THE H^+ AND OH^- CONCENTRATIONS WHICH TAKE PLACE IN THE FORMATION OF CERTAIN PROTEIN COMPOUNDS*

It has been known for some time that certain proteins combine with other proteins to form compound proteins. Kossel⁹³ states that the protamines in weakly alkaline solutions combine with proteins to form compounds which precipitate under proper conditions. Kutscher⁹⁴ working with albumoses obtained from Witte's peptone prepared compounds not only with protamine but also with various other proteins. While studying the properties of the histones, Bang⁹⁵ was able to confirm the observations of Kutscher and further showed that various members of this group combine with proteins to form compounds. Kossel and Kutscher⁹⁶ surmised that histones (on account of the high content of basic substances) might be a combination of protamine with other proteins, but later Kossel and Pringle⁹⁷ showed that this view was incorrect since in the first step in the peptic digestion of histones histopeptone is formed, whereas protamine-protein combinations are split into protamine and protein. Hunter⁹⁸ prepared a number of compounds of protamine with various proteins, such combinations as clupein-casein, clupein-gelatin and clupein-edestin being obtained. He further determined the proportion of the two substances which unite to form the compound protein. Gay and Robertson^{92a} studying the antigenic properties of compound proteins, prepared the compound protamine (salmin) caseinate. Robertson,^{87c} unaware of the work of Bang,

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surmised from the possible relation of the histones to the protamines that globin might also unite with other proteins to form compounds. He prepared globin caseinate and determined its refractive index in N/10 KOH solution. Later Gay and Robertson^{92b} studied its antigenic properties.

It will be noted that in the work recorded precipitation of the compound took place in neutral or alkaline solutions, although Kossel⁹³ states that a nuclein may be formed in acid solution from the combination of nucleic acid and protein. Altmann⁹⁹ had previously observed that proteins could combine in acid solution and Milroy¹⁰⁰ prepared a number of protein-nucleic acid compounds. No observations have apparently been made to determine the true acidity (or alkalinity) of the protein solutions used or of the mixture when precipitation of the compound took place. The conditions for precipitation must be such that salting out of either of the proteins by inorganic salts formed during the reaction must not take place and the acidity of the solution must be such that the compound protein, if formed, will not be dissolved.

In the present work use of the gas-chain has been made to follow the change of acidity (or alkalinity) of the solution during the precipitation of a compound protein to distinguish, if possible, salting out of either protein from true compound precipitation. Determination of the exact hydrogen ion concentration serves as a criterion for the duplication of any work in the preparation of compound proteins, since it appears that the exact acidity (or alkalinity) is a very important factor in the preparation of such compounds.

Use of the hydrogen electrode has been made by a large number of observers. Böttger¹⁰¹ made use of the gas-chain to determine the neutral point in the titration of acids and bases. The use of the hydrogen electrode for such purposes has been emphasized by Hildebrand,¹⁰² who points out its many applications in the analytical field. Salm,¹⁰³ determining the H^+ concentrations in various phosphate mixtures, was able to prepare a scale of indicators for the colorimetric estimation of H^+ and OH^- concentrations. Schmidt and Finger¹⁰⁴ showed that solutions of definite H^+ and OH^- concentrations may be made by

using mixtures of various borates. They also point out that from the shape of the titration curve the existence of a compound in solution may be determined.^{104a} Thus they confirmed observations made by various other methods by Noyes and Whitney,¹⁰⁵ Kahlenberg and Schreiner,¹⁰⁶ and Shelton¹⁰⁷ that a compound NaH_2BO_3 , or the anhydride NaBO_2 , or the corresponding ions H_2BO_3 and BO_2 exist in solution. Tammann¹⁰⁸ and others have made use of cooling curves to determine the existence of compounds in alloys. In the field of protein chemistry much use has also been made of the hydrogen electrode. Thus Robertson¹⁰⁹ used it to determine the dissociation of serum globulin at varying hydrogen ion concentrations, and the dissociation of potassium caseinate¹¹⁰ and ovomucoid¹¹¹ at varying alkalinities. Robertson and Schmidt¹¹² were able to follow the change of alkalinity which took place during the progress of tryptic digestions of certain proteins. The many uses of the hydrogen electrode are set forth in monographs by Michaelis¹¹³ and Sørensen.^{113a}

The plan of these experiments was to determine the H^+ and OH^- concentrations in a solution of protein *a*. Definite amounts of either a solution of protein *b* or an inorganic salt used to precipitate protein *a* from its solution, were then added to the solution of protein *a*. The H^+ concentration was then determined in the solution of protein *a* after each addition of the solution of protein *b*. A titration curve can thus be plotted, using cubic centimeters of solution *b* as ordinates and the resultant H^+ or OH^- concentration as abscissae. In this way a titration curve is obtained similar to those described by Böttger,¹⁰¹ Schmidt and Finger,¹⁰⁴ and Hildebrand.¹⁰²

The method used to determine the H^+ concentrations was essentially the same as that used by Robertson,¹⁰⁹ and Robertson and Schmidt,¹¹² and described by Schmidt and Finger,¹⁰⁴ and Robertson.¹¹⁴ Hydrogen was generated by the electrolysis of a 6 per cent (by volume) solution of concentrated H_2SO_4 and passed over heated platinized asbestos to rid it of any oxygen or oxygen compounds. The hydrogen then passed through the solution at two points, through a nozzle at the bottom of the cell and through a Cottrell gauze electrode.¹¹⁵ In this way the three phases, platinum, hydrogen, and solution were brought

into intimate contact. A calomel electrode,¹¹⁶ using N/10 KCl, was used as the other extremity of the chain. The apparatus is shown in figure 1. *A* is the gauze electrode dipping into the solution contained in cell *B*, *D* a beaker containing N/10 KCl, and *F* the calomel electrode. Connection with cell *B* is made by means of an agar tube saturated with KCl, which eliminates contact potential.^{117*} To eliminate contamination of the solution in cell *B*, the stop-cock connecting the side arm with the cell was kept closed except during the measurement of the potential, and likewise the agar tube was dipped into the solution in the side

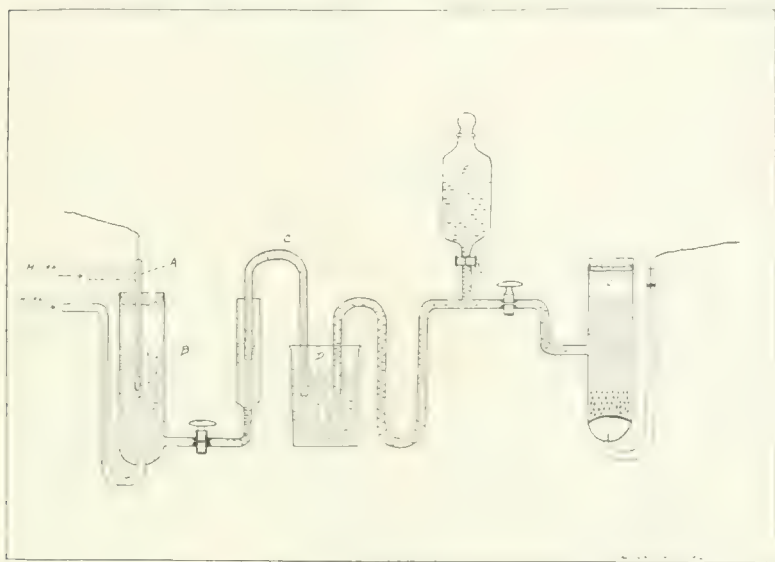


Figure 1

- A*, Platinum gauze electrode. *D*, Beaker with N/10 KCl.
B, Titration cell. *E*, Separatory funnel with N/10 KCl.
C, Agar tube saturated with KCl. *F*, N/10 KCl-HgCl-Hg electrode.

arm only while readings were being taken. Through a hole in the stopper of cell *B* any amount of a solution *b* could be introduced. Measurement of the potential was made on a 100-centimeter bridge, using a sensitive Leeds and Northrup galvanometer (kindly loaned by the Physics Department) as zero instrument. Three Edison-Lalande cells connected in series fur-

* This may not be strictly true, but since we are not concerned with absolute values but merely with changes in voltage, this factor may be neglected.

nished current through the potentiometer wire and the E. M. F. was checked against a Weston cell after each reading. The protein solutions *a* and *b*, as well as the gauze electrode were separately saturated with hydrogen before bringing them together, the necessity for this having been shown by Robertson¹¹⁹ and by Desha and Acree.¹¹⁸ Hydrogen was allowed to bubble through the solution from forty-five to sixty minutes before determining the E. M. F., and this was likewise done after each addition of solution *b*. All determinations were made at room temperature. To prevent foaming a few drops of octyl alcohol were floated on the surface of the solution in *B* after the introduction of the electrode. The H^+ and OH^- concentrations corresponding to the E. M. F.'s were taken from tables previously calculated by Schmidt.¹¹⁹

Since it is not feasible to determine H^+ or OH^- concentrations in ammoniacal solutions by means of the gas-chain, the experiments were made with compounds of globin. As stated by Bang⁹⁵ and Robertson,^{87c} precipitation of the compound protein takes place in slight excess of NaOH or KOH. Globin was prepared according to a modification of the method described by Robertson.¹²⁰ Two preparations were made, one precipitated by NH_4Cl from an ammoniacal solution and the other by alcohol and ether from HCl solution. These correspond to Preparations I and II described by Robertson. Preparation II is soluble in water without addition of acid or alkali. The casein employed was Eimer and Amend's ("nach Hammarsten") which had been further purified according to Robertson.¹²¹ Deuteroalbumose was made from Witte's peptone as described by Kutscher.⁹⁴ The nucleic acid was a preparation extracted by Dr. A. E. Taylor from the sperm of the Pacific Coast salmon (probably according to the method of Altmann⁹⁹). Bile salt was prepared from ox bile according to the method of Plattner.¹²²

The following is a tabular representation of the results obtained in the experiment. In the first column are given the volumes of the titrating solution *b* which were added to a given amount of solution *a*. The second column shows the E. M. F. determined after the establishment of equilibrium. The H^+ and OH^- concentrations corresponding to the E. M. F.'s are given in the next two columns. In the last column are given the

calculated possible errors in determining the H^+ concentration on the assumption that the determination of the E. M. F.'s was accurate to a millivolt.

Curves showing either the change in the H^+ or the OH^- concentration which took place during the titration as determined by experiment are plotted and given below. These will be referred to as titration curves. The first experiments were carried out to determine what effect was produced on the H^+ concentration by simple dilution and by salting out with those salts which might be formed as intermediate products of the reaction; so that, if possible, a distinction between salting out and true protein compound formation might be made. The results are given in tables I, II, and III and are shown graphically in figure 2. The change in H^+ concentration is a direct

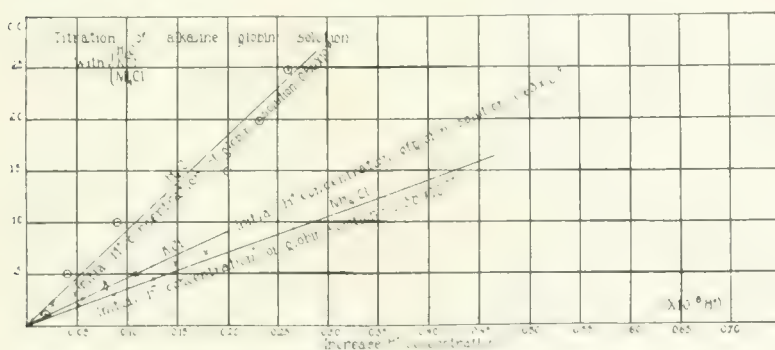


Figure 2

TABLE I

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH WATER

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c.

0.1 N KOH, then titrated back with 7 c.c. 0.1 N HCl.

50 c.c. globin solution titrated. No precipitate produced.

H ₂ O added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	.861	0.80×10^{-9}	0.80×10^{-5}	$\pm 0.06 \times 10^{-9}$
1	.856	$.98 \times 10^{-9}$	$.65 \times 10^{-5}$	$.08 \times 10^{-9}$
5	.850	$.12 \times 10^{-8}$	5.3×10^{-6}	$.01 \times 10^{-8}$
10	.842	$.17 \times 10^{-8}$	3.8×10^{-6}	$.01 \times 10^{-8}$
15	.830	$.28 \times 10^{-8}$	2.3×10^{-6}	$.03 \times 10^{-8}$
20	.827	$.31 \times 10^{-8}$	2.1×10^{-6}	$.02 \times 10^{-8}$
25	.825	$.34 \times 10^{-8}$	1.9×10^{-6}	$.03 \times 10^{-8}$

TABLE II

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH A SATURATED SOLUTION OF KCl.

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c. 0.1 N KOH, then titrated back with 7 c.c. 0.1 N HCl.
50 c.c. globin solution titrated. Precipitate produced.

KCl solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.867	0.63×10^{-9}	1.0×10^{-5}	$\pm 0.05 \times 10^{-9}$
1	.859	$.87 \times 10^{-9}$	$.74 \times 10^{-5}$	$.07 \times 10^{-9}$
2	.859	$.87 \times 10^{-9}$	$.74 \times 10^{-5}$	$.07 \times 10^{-9}$
3	.853	$.11 \times 10^{-8}$	5.8×10^{-6}	$.01 \times 10^{-8}$
4	.847	$.14 \times 10^{-8}$	4.6×10^{-6}	$.01 \times 10^{-8}$
5	.842	$.17 \times 10^{-8}$	3.8×10^{-6}	$.01 \times 10^{-8}$
6	.837	$.21 \times 10^{-8}$	3.1×10^{-6}	$.02 \times 10^{-8}$
7	.834	$.24 \times 10^{-8}$	2.7×10^{-6}	$.02 \times 10^{-8}$

TABLE III

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH A 0.25 PER CENT SOLUTION OF NH₄Cl.

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c. 0.1 N KOH, then titrated back with 7 c.c. 0.1 N HCl.
50 c.c. globin solution titrated. Precipitate produced.

NH ₄ Cl solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.927	0.58×10^{-10}	1.1×10^{-4}	$\pm 0.05 \times 10^{-10}$
.5	.911	$.11 \times 10^{-9}$	5.8×10^{-5}	$.01 \times 10^{-9}$
1.0	.893	$.22 \times 10^{-9}$	2.9×10^{-5}	$.01 \times 10^{-9}$
1.5	.874	$.48 \times 10^{-9}$	1.3×10^{-5}	$.04 \times 10^{-9}$
2.0	.869	$.58 \times 10^{-9}$	1.1×10^{-5}	$.04 \times 10^{-9}$
2.5	.868	$.61 \times 10^{-9}$	1.1×10^{-5}	$.05 \times 10^{-9}$
3.0	.857	$.94 \times 10^{-9}$	$.68 \times 10^{-5}$	$.07 \times 10^{-9}$
3.5	.859	$.87 \times 10^{-9}$	$.74 \times 10^{-5}$	$.07 \times 10^{-9}$
4.5	.848	$.13 \times 10^{-8}$	4.9×10^{-6}	$.01 \times 10^{-8}$
5.5	.843	$.17 \times 10^{-8}$	3.9×10^{-6}	$.02 \times 10^{-8}$
7.5	.835	$.23 \times 10^{-8}$	2.8×10^{-6}	$.02 \times 10^{-8}$
9.5	.831	$.27 \times 10^{-8}$	2.4×10^{-6}	$.03 \times 10^{-8}$
14.5	.819	$.43 \times 10^{-8}$	1.5×10^{-6}	$.03 \times 10^{-8}$

function of the titrating solution and the curves are straight lines, despite the fact that with both NH₄Cl and KCl a precipitate is obtained. In these instances we apparently have either a purely physical phenomenon or a chemical one which

involves no sudden change in the H^+ concentration such as to give a break in the curve. The precipitation of proteins by inorganic salts has been studied extensively by Hardy,⁵⁰ Pauli,⁵¹ Hofmeister,⁵² Galeotti,⁵³ and many others, and is discussed at length by Robertson.¹¹⁴

The titration of globin in acid and alkali solutions by alkali and acid respectively was next studied. For this purpose, Preparation I, dissolved by a small amount of alkali, and Preparation II, which is water-soluble and acid in reaction, were used. During the titration the protein was precipitated and redissolved by excess of acid or alkali respectively. The results are given in tables IV and V and the changes in H^+ and OH^- concentrations graphically represented in figures 3 and 4. Since these reactions involve direct changes in H^+ and OH^- concentrations and true compounds between protein and acid and alkali are formed, we obtain curves differing from those in which salting out of protein has taken place. The titration curves of acid and

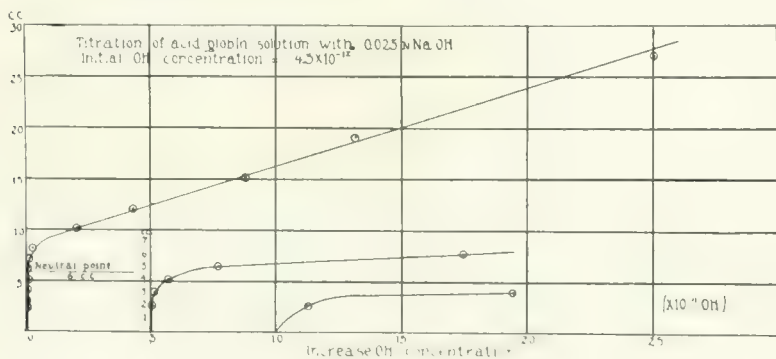


Figure 3

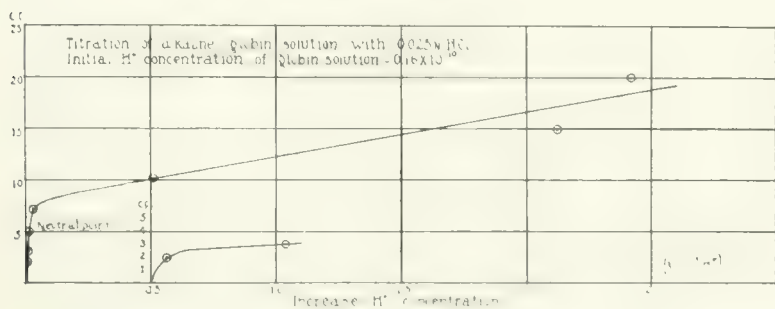


Figure 4

TABLE IV

TITRATION OF ACID GLOBIN (PREPARATION II) SOLUTION WITH
0.025 N NaOH.Concentration of globin, 0.2%. Globin dissolved in water without
addition of acid or alkali.50 c.c. globin solution titrated. Precipitate produced which
dissolved in excess of alkali.

NaOH solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.499	0.15×10^{-2}	4.3×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	.534	$.37 \times 10^{-3}$	1.7×10^{-11}	$.02 \times 10^{-3}$
3	.578	$.64 \times 10^{-4}$	1.0×10^{-10}	$.04 \times 10^{-4}$
4	.632	$.75 \times 10^{-5}$	$.86 \times 10^{-9}$	$.03 \times 10^{-5}$
5	.662	$.23 \times 10^{-5}$	2.8×10^{-9}	$.01 \times 10^{-5}$
6	.757	$.51 \times 10^{-7}$	1.3×10^{-7}	$.02 \times 10^{-7}$
7	.840	$.19 \times 10^{-8}$	3.5×10^{-6}	$.01 \times 10^{-8}$
8	.893	$.22 \times 10^{-9}$	2.9×10^{-5}	$.01 \times 10^{-9}$
10	.941	$.33 \times 10^{-10}$	2.0×10^{-4}	$.01 \times 10^{-10}$
12	.961	$.15 \times 10^{-10}$	4.3×10^{-4}	$.01 \times 10^{-10}$
15	.979	$.72 \times 10^{-11}$	$.89 \times 10^{-3}$	$.03 \times 10^{-11}$
19	.989	$.48 \times 10^{-11}$	1.3×10^{-3}	$.02 \times 10^{-11}$
27	1.005	$.26 \times 10^{-11}$	2.5×10^{-3}	$.01 \times 10^{-11}$

TABLE V

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH
0.025 N HCl.Concentration of globin, 0.2%. Globin dissolved in 2 c.c. 0.1 N NaOH,
titrated back with 1.5 c.c. 0.1 N HCl.50 c.c. globin solution used for titration. Precipitate produced which
dissolved in excess of acid.

HCl solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.959	0.16×10^{-10}	4.0×10^{-4}	$\pm 0.01 \times 10^{-10}$
2	.920	$.76 \times 10^{-10}$	$.84 \times 10^{-4}$	$.03 \times 10^{-10}$
3	.870	$.56 \times 10^{-9}$	1.2×10^{-5}	$.02 \times 10^{-9}$
5	.747	$.76 \times 10^{-7}$	$.85 \times 10^{-7}$	$.03 \times 10^{-7}$
7	.609	$.19 \times 10^{-4}$	3.4×10^{-10}	$.02 \times 10^{-4}$
10	.525	$.53 \times 10^{-3}$	1.2×10^{-11}	$.02 \times 10^{-3}$
15	.490	$.22 \times 10^{-2}$	3.0×10^{-12}	$.01 \times 10^{-2}$
20	.487	$.24 \times 10^{-2}$	2.6×10^{-12}	$.01 \times 10^{-2}$

alkaline globin are very similar to the titration curve of hydrochloric acid by sodium hydroxide given by Böttger¹⁰¹ and cited by Hildebrand,¹⁰² except that a different neutral point is obtained.

This is to be expected, since globin can act either as an acid or a base. Thus in titrating acid globin with alkali, on approaching the neutral point, globin will become acid and will neutralize alkali. The sudden shift in the slope of the curve near the neutral point indicates compound formation and serves as a criterion for the determination of compound formation, whether the compound is precipitated or remains in solution. This will be shown even better in other curves. That the first part of the curve is not a straight line but appears to be so since the changes

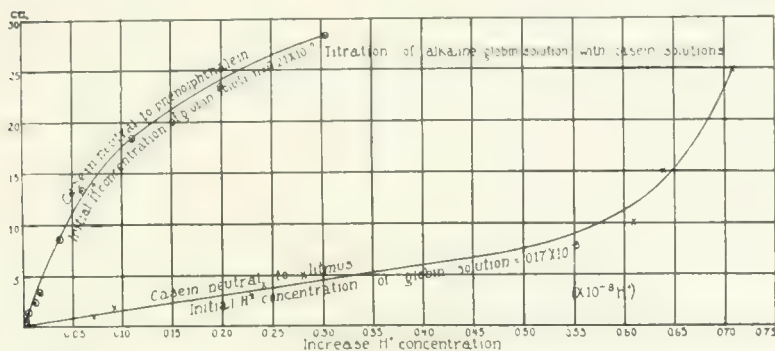


Figure 5

TABLE VI

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH A SOLUTION OF CASEIN (NEUTRAL TO PHENOLPHTHALEIN).

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c. 0.1 N KOH, then titrated back with 6.9 c.c. 0.1 N HCl. Concentration of casein in titrating solution, 0.5%. 0.25 gm. casein dissolved by 4 c.c. 0.1 N KOH, then titrated back with 2 c.c. 0.1 N HCl. 50 c.c. globin solution titrated. No precipitate produced.

Casein solution added c.c.	E. M. F. volts	Conc. H^+	Conc. OH^-	Possible error in determining Conc. H^+
0	0.894	0.21×10^{-9}	3.0×10^{-5}	$\pm 0.02 \times 10^{-9}$
.5	.892	$.23 \times 10^{-9}$	2.8×10^{-5}	$.02 \times 10^{-9}$
1.5	.888	$.27 \times 10^{-9}$	2.4×10^{-5}	$.02 \times 10^{-9}$
2.5	.883	$.33 \times 10^{-9}$	1.9×10^{-5}	$.02 \times 10^{-9}$
3.5	.880	$.38 \times 10^{-9}$	1.7×10^{-5}	$.03 \times 10^{-9}$
8.5	.869	$.58 \times 10^{-9}$	1.1×10^{-5}	$.04 \times 10^{-9}$
13.5	.861	$.80 \times 10^{-9}$	$.80 \times 10^{-5}$	$.06 \times 10^{-9}$
18.5	.848	$.13 \times 10^{-8}$	1.8×10^{-6}	$.01 \times 10^{-8}$
23.5	.836	$.22 \times 10^{-8}$	3.0×10^{-6}	$.02 \times 10^{-8}$
28.5	.825	$.34 \times 10^{-8}$	1.9×10^{-6}	$.03 \times 10^{-8}$

TABLE VII

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH A SOLUTION OF CASEIN (NEUTRAL TO LITMUS).

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c. 0.1 N KOH, then titrated back with 7.1 c.c. 0.1 N HCl.

50 c.c. globin solution titrated. Concentration of casein in titrating solution, 0.5%. 0.25 gm. casein dissolved by 3 c.c. 0.1 N KOH, then titrated back with 1.5 c.c. 0.1 N HCl. Slight precipitate produced.

Casein solution added c.c.	E. M. F. volts	Conc. H^+	Conc. OH^-	Possible error in determining Conc. H^+
0	0.843	0.17×10^{-8}	3.9×10^{-6}	$\pm 0.02 \times 10^{-8}$
1	.833	$.24 \times 10^{-8}$	2.6×10^{-6}	$.01 \times 10^{-8}$
2	.831	$.26 \times 10^{-8}$	2.4×10^{-6}	$.02 \times 10^{-8}$
3	.821	$.40 \times 10^{-8}$	1.6×10^{-6}	$.03 \times 10^{-8}$
4	.820	$.41 \times 10^{-8}$	1.6×10^{-6}	$.03 \times 10^{-8}$
5	.818	$.45 \times 10^{-8}$	1.4×10^{-6}	$.04 \times 10^{-8}$
10	.804	$.78 \times 10^{-8}$	$.82 \times 10^{-6}$	$.06 \times 10^{-8}$
15	.803	$.81 \times 10^{-8}$	$.79 \times 10^{-6}$	$.06 \times 10^{-8}$
25	.801	$.88 \times 10^{-8}$	$.73 \times 10^{-6}$	$.07 \times 10^{-8}$

in H^+ or OH^- concentrations are small as compared with the rest of the curve, is shown on magnifying the first portion of the titration curve.

As previously stated, both Bang and Robertson obtained compounds of globin and casein which they state were precipitated in alkaline solution. On attempting to duplicate this work, no precipitate was obtained, though a compound between globin and casein is formed as shown by the data in tables VI and VII and represented graphically in figure 5. The compound is apparently soluble. However, on titrating an acid solution of globin with a solution of casein (neutral to phenolphthalein) a precipitate was obtained and the titrating curve (see data in table VIII and figure 6) shows that a compound was formed. Apparently Robertson had used Globin Preparation II, which, being precipitated from an acid solution by alcohol and ether, was the acid compound of globin; and by dissolving in weak alkali, Robertson had merely partly neutralized the acid combined with the globin (not enough to cause precipitation of the globin) so that his solution was still acid, instead of alkaline as one is led to believe from his description. This was likewise true of the work of

Bang, who prepared globin according to the method of Schulz.¹¹ Determination of the initial acidity of the globin solution would have served as a criterion for the duplication of the work.

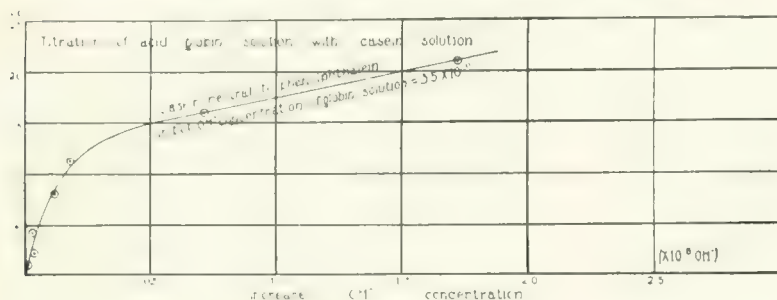


Figure 6

TABLE VIII
TITRATION OF ACID GLOBIN (PREPARATION II) SOLUTION WITH A
SOLUTION OF CASEIN.

Concentration of globin, 0.2%. Globin dissolved in water and 1 c.c. 0.1 N NaOH added. Concentration of casein (neutral to phenolphthalein), 0.5%. 50 c.c. globin solution titrated. Precipitate produced.

Casein solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.552	0.18×10^{-3}	3.5×10^{-11}	$\pm 0.01 \times 10^{-3}$
1	.577	$.67 \times 10^{-4}$	$.96 \times 10^{-10}$	$.03 \times 10^{-4}$
2	.609	$.19 \times 10^{-4}$	3.4×10^{-10}	$.01 \times 10^{-4}$
4	.614	$.15 \times 10^{-4}$	4.2×10^{-10}	$.01 \times 10^{-4}$
6	.623	$.11 \times 10^{-4}$	6.0×10^{-10}	$.01 \times 10^{-4}$
8	.637	$.61 \times 10^{-5}$	1.1×10^{-9}	$.03 \times 10^{-5}$
11	.650	$.36 \times 10^{-5}$	1.8×10^{-9}	$.02 \times 10^{-5}$
16	.685	$.90 \times 10^{-6}$	$.71 \times 10^{-8}$	$.04 \times 10^{-6}$
21	.707	$.37 \times 10^{-6}$	1.7×10^{-8}	$.03 \times 10^{-6}$

Globin also forms compounds with nucleic acid and taurocholic acid, precipitation taking place in an acid solution. The titration curve of globin with nucleic acid (see table IX and figure 7) is very similar to that obtained on titration of acid globin with casein. The titration curve of acid globin with bile salts (see tables X and XI, and figure 8) shows a sharp break at the point where the compound protein starts to precipitate, and the slope of the curve changes. This is to be expected since

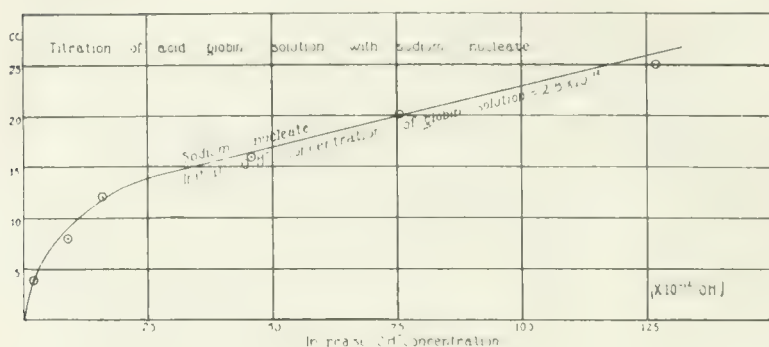


Figure 7

TABLE IX

TITRATION OF ACID GLOBIN (PREPARATION II) SOLUTION WITH A SOLUTION OF SODIUM NUCLEATE.

Concentration of globin, 0.2%. Globin dissolved in water without addition of acid or alkali. Concentration of nucleic acid, 0.4%. Nucleic acid previously neutralized by NaOH. 50 c.c. globin solution titrated. Precipitate produced.

Sodium nucleate solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	0.488	0.23×10^{-2}	2.8×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	.486	$.25 \times 10^{-2}$	2.5×10^{-12}	$.01 \times 10^{-2}$
4	.504	$.12 \times 10^{-2}$	5.2×10^{-12}	$.01 \times 10^{-2}$
8	.525	$.53 \times 10^{-3}$	1.2×10^{-11}	$.02 \times 10^{-3}$
12	.536	$.34 \times 10^{-3}$	1.9×10^{-11}	$.02 \times 10^{-3}$
16	.560	$.13 \times 10^{-3}$	4.9×10^{-11}	$.01 \times 10^{-3}$
20	.572	$.82 \times 10^{-4}$	$.78 \times 10^{-10}$	$.03 \times 10^{-4}$
25	.585	$.49 \times 10^{-4}$	1.3×10^{-10}	$.03 \times 10^{-4}$

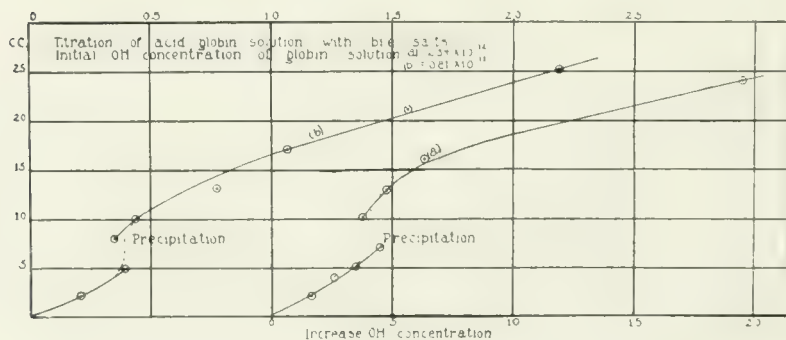


Figure 8

TABLE X
TITRATION OF ACID GLOBIN (PREPARATION II) SOLUTION WITH A
SOLUTION OF BILE SALTS

Concentration of globin, 0.2%. Globin dissolved in water without addition of acid or alkali. Concentration of bile salts in aqueous solution, 0.4%. Precipitate produced.

Bile salts solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	.484	0.27×10^{-2}	2.3×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	.498	$.16 \times 10^{-2}$	4.1×10^{-11}	$.01 \times 10^{-2}$
4	.503	$.13 \times 10^{-2}$	5.0×10^{-12}	$.01 \times 10^{-2}$
5	.507	$.11 \times 10^{-2}$	5.9×10^{-12}	$.01 \times 10^{-2}$
7	.511	$.93 \times 10^{-3}$	$.69 \times 10^{-11}$	$.04 \times 10^{-3}$
10	.508	$.11 \times 10^{-2}$	6.1×10^{-12}	$.01 \times 10^{-2}$
13	.512	$.89 \times 10^{-3}$	$.72 \times 10^{-11}$	$.04 \times 10^{-3}$
16	.517	$.73 \times 10^{-3}$	$.88 \times 10^{-11}$	$.03 \times 10^{-3}$
24	.540	$.29 \times 10^{-3}$	2.2×10^{-11}	$.01 \times 10^{-3}$

TABLE XI
TITRATION OF ACID GLOBIN (PREPARATION II) SOLUTION WITH A
SOLUTION OF BILE SALTS

Concentration of globin, 0.2%. Globin dissolved in water without addition of acid or alkali. Concentration of bile salts in aqueous solution, 0.4%. Several drops 0.1 N NaOH added to the solution of bile salts to make it slightly alkaline. Precipitate produced during titration.

Bile salts solution added c.c.	E. M. F. volts	Conc. H ⁺	Conc. OH ⁻	Possible error in determining Conc. H ⁺
0	.515	0.79×10^{-3}	$.81 \times 10^{-11}$	$\pm 0.04 \times 10^{-3}$
2	.521	$.63 \times 10^{-3}$	1.0×10^{-11}	$.03 \times 10^{-3}$
5	.525	$.53 \times 10^{-3}$	1.2×10^{-11}	$.02 \times 10^{-3}$
8	.524	$.55 \times 10^{-3}$	1.2×10^{-11}	$.02 \times 10^{-3}$
10	.526	$.51 \times 10^{-3}$	1.3×10^{-11}	$.02 \times 10^{-3}$
13	.532	$.40 \times 10^{-3}$	1.6×10^{-11}	$.02 \times 10^{-3}$
17	.536	$.34 \times 10^{-3}$	1.9×10^{-11}	$.02 \times 10^{-3}$
21	.542	$.27 \times 10^{-3}$	2.4×10^{-11}	$.01 \times 10^{-3}$
25	.548	$.21 \times 10^{-3}$	3.0×10^{-11}	$.01 \times 10^{-3}$

we are titrating with a mixture of two salts, sodium taurocholate and sodium glycocholate. Globin also forms a compound with deuterioalbumose, the latter acting as a weak acid. The titration curve (figure 9 and table XII) indicates the formation of a compound, the proportion being roughly two parts of globin to one of albumose.

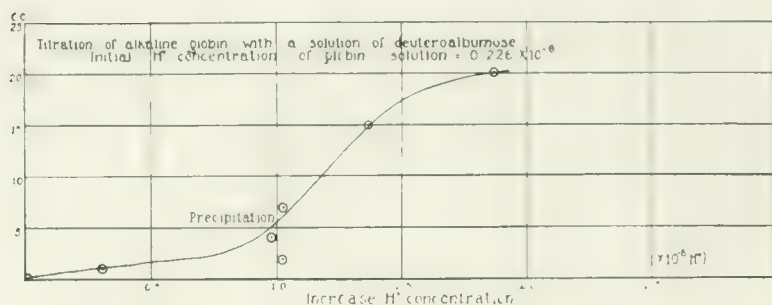


Figure 9

TABLE XII

TITRATION OF ALKALINE GLOBIN (PREPARATION I) SOLUTION WITH A SOLUTION OF DEUTOALBUMOSE

Concentration of globin, 0.5%. 0.25 gm. globin dissolved by 8 c.c. 0.1 N KOH, then titrated back with 7.1 c.c. 0.1 N HCl. Concentration of deutoalbumose, 0.6%. 50 c.c. globin solution titrated. Precipitate produced.

Albumose solution added c.c.	E. M. F. volts	Conc. H^+	Conc. OH^-	Possible error in determining Conc. H^+
0	0.835	0.23×10^{-8}	2.8×10^{-6}	$\pm 0.02 \times 10^{-8}$
1	.813	$.54 \times 10^{-8}$	1.2×10^{-6}	$.04 \times 10^{-8}$
2	.792	$.13 \times 10^{-7}$	5.1×10^{-7}	$.01 \times 10^{-7}$
4	.793	$.12 \times 10^{-7}$	5.3×10^{-7}	$.01 \times 10^{-7}$
7	.792	$.13 \times 10^{-7}$	5.1×10^{-7}	$.01 \times 10^{-7}$
15	.786	$.16 \times 10^{-7}$	4.0×10^{-7}	$.01 \times 10^{-7}$
20	.779	$.21 \times 10^{-7}$	3.0×10^{-7}	$.01 \times 10^{-7}$

II

ANTIGENIC PROPERTIES OF CERTAIN COMPOUND PROTEINS

The antigenic properties of two compound proteins—protamine edestinate and globin-albumose—were studied. These were prepared as follows:

Protamine sulphate.—In the preparation of this substance the method of Kossel,¹²⁷ used by Taylor,¹²⁸ Robertson,¹²⁹ and others was followed. The ripe testicles of the Pacific salmon were minced and the macerated mass which was thus obtained was shaken up in tall glass cylinders with five or six times its volume of distilled water. The thick suspension of sperm which was

thus obtained was syphoned off from the supernatant connective tissue, strained through cheesecloth, and curdled by the addition of about 80 c.c. of M/3 acetic acid. The curdled mass of sperm was then washed in ten times its volume of 95 per cent alcohol, repeating the washing twice; it was then washed twice in the same volume of absolute ether. The ether-wet mass was then transferred to the incubator and dried over H_2SO_4 at 37°C .

Each fifteen grams of the dried sperm was stirred up in 350 c.c. of 1 per cent (by volume) of conc. H_2SO_4 for about six hours. The mixture was then filtered through hardened filter paper and the filtrate obtained from the extraction of fifteen grams of sperm was placed in a tall glass cylinder of about four litres capacity, which was filled with 95 per cent alcohol. After allowing the precipitate to settle, the supernatant fluid was syphoned off, the precipitate contained in several cylinders was collected into one and this was filled with absolute alcohol, allowed to settle, the alcohol syphoned off, and the precipitate collected on hardened filter paper. The precipitates from about 300 grams of sperm were dissolved by the addition of about four litres of hot water, (80°C) filtered, and reprecipitated by the addition of ten volumes of 95 per cent alcohol. The precipitate was washed twice by decantation with absolute alcohol and twice again with absolute ether. After syphoning off the ether, the precipitate was collected on a hardened filter paper and dried over H_2SO_4 in an incubator at 37°C . The product is an amorphous white powder, which is easily soluble in water, forming a clear solution. According to Taylor¹²⁸ it has the formula $\text{C}_{100}\text{H}_{177}\text{O}_{17}\text{N}_{17} \cdot 2\text{H}_2\text{SO}_4$.

Edestin was prepared from hemp seed (*Cannabis sativa*) according to the method of Osborne¹²⁹ as follows: Selected hemp seed was thoroughly ground and extracted with ordinary ether in an extractor already described by the author,¹³¹ to remove fat and chlorophyll. This was reground several times, re-extracted and finally sieved. The fat-free meal was macerated with ten times the amount of 5 per cent NaCl, expressed in cubic centimeters as the weight of meal taken, kept at 60°C for one hour and filtered in an oven at 40°C . The filtrate was allowed to stand in the refrigerator over night, causing crystals of edestin

to separate. The supernatant fluid was syphoned off and the precipitate redissolved in warm NaCl solution, and again precipitated as before. This was again repeated. The precipitate was then washed by decantation with CO₂-free distilled water, keeping the temperature at 5° C. The decantation was repeated with 50 per cent alcohol, then with 95 per cent alcohol, and finally with absolute alcohol and ether, and dried over H₂SO₄ in an incubator at 37° C. The product is a light, white, crystalline powder. According to Osborne it has the composition C = 51.3, H = 6.8, N = 18.8, S = .9, O = 22.2 and the molecular weight is 14,500. This preparation was also used to determine the refractive index constant.¹³²

Protamine edestinate was prepared essentially according to Hunter⁹⁸ as follows: One gram of edestin was dissolved in 20 c.c. of N/10 NH₄OH and 15 c.c. of 5 per cent NaCl, filtered, and to the filtrate 150 c.c. water containing 7 c.c. of 25 per cent (by volume) of concentrated NH₄OH was added. One gram of protamine sulphate was dissolved in 100 c.c. N/10 NH₄OH and 150 c.c. 5 per cent NaCl. On mixing the two solutions, a white flocculent precipitate was formed which readily settled. This precipitate was washed twice by decanting with a solution of NH₄OH (1 per cent by volume of conc. NH₄OH), then twice with absolute alcohol and twice with absolute ether and dried over H₂SO₄ in an incubator at 37° C. The substance is a white amorphous powder, soluble in N/10 NaOH. From the nitrogen determination, the compound contains nine parts of edestin to one of protamine.

Globin was prepared according to Robertson's¹²⁰ modification of the method of Schulz as follows: Fresh defibrinated ox-blood was centrifuged and the supernatant serum removed by suction. The suspension of corpuscles was diluted to the original volume of blood by addition of M/6 NaCl solution, centrifuged, and the supernatant fluid removed by suction. This was repeated six times. After the last centrifugalization (half an hour) as much supernatant fluid as possible was removed. The thick suspension of corpuscles was laked by addition of ten times its volume of distilled water and allowed to stand over night to allow stromata, etc., to settle. Only the upper half of the fluid in the cylinders

was used for the subsequent work. To each litre of the corpuscle solution was added 22 c.c. of concentrated HCl (Sp. G. 1.18), the mixture shaken and one litre of ether added (note that the proper amount of acid to be added at this point is important; it should be determined for each lot of haemoglobin solution) and the mixture again shaken. On centrifuging (slow speed) two layers were formed, an upper, jelly-like, dark-colored emulsion, and a lower, brown, aqueous solution. The upper layer was removed by suction and carefully shaken several times with a 25 per cent (by volume) solution of alcohol in ether, the lower layer being removed in a separatory funnel after each addition of the alcohol-ether mixture. (During the process of extraction by ether, care must be taken to prevent emulsification. The mixtures should not be shaken too hard.) The aqueous layer was diluted to ten times its volume by distilled water and concentrated NH_4OH added to just redissolve the precipitate which is formed. On nearly neutralizing this ammonia with HCl, globin was again precipitated. After standing over night, the precipitate was washed successively with distilled water, 95 per cent alcohol, absolute alcohol, and ether and dried over H_2SO_4 in an incubator at 37°C . The product is a light, yellowish, amorphous powder, soluble in N/10 HCl or N/10 NaOH.

Deuteroalbumose was prepared from Witte's peptone according to the method of Kutscher⁹⁴ as follows: To 100 grams of Witte's peptone were added 800 c.c. of 2 per cent HCl and the mixture heated in an Arnold sterilizer for six hours. The hot solution was saturated with NaCl and allowed to stand over night, causing a black, gummy substance to separate, which was filtered off. The filtrate was made neutral to litmus with NaOH and saturated while boiling with $(\text{NH}_4)_2\text{SO}_4$. From the boiling mixture, deuteroalbumose slowly separates and can be removed with a stirring rod. The deuteroalbumose was redissolved in distilled water and again precipitated by $(\text{NH}_4)_2\text{SO}_4$. To remove $(\text{NH}_4)_2\text{SO}_4$, a slight excess of $\text{Ba}(\text{OH})_2$ was added to the aqueous solution of deuteroalbumose, and the BaSO_4 removed by filtration. The filtrate was concentrated and the slight excess of barium precipitated as the carbonate (by passing CO_2 through the solution), and filtered off. On addition of a large volume of

95 per cent alcohol, deuteroalbumose was precipitated in large floccules which settle very quickly. The precipitate was further washed by decanting with large volumes of absolute alcohol and ether respectively, filtered and dried over H_2SO_4 in an incubator at 37°C . The product is a slightly colored light powder very soluble in water.

Globin-albumose compound was prepared as follows: Ten grams of globin were dissolved in 50 c.c. of N/10 NaOH and diluted to two litres. N/10 HCl was then added to neutralize the excess alkali without precipitating the globin. This gives an alkalinity of about $3 \times 10^{-6} \text{OH}^-$. Five grams of deuteroalbumose were dissolved in a litre of distilled water and added to the solution of globin. A precipitate is immediately formed which quickly settles. This was successively washed by decanting with distilled water, 95 per cent alcohol, absolute alcohol and absolute ether, filtered and dried over H_2SO_4 in an incubator at 37°C . The product is a light-colored amorphous powder, soluble in alkali. That a true compound is formed is shown by the previously cited titration curve.

FIXATION EXPERIMENTS WITH PROTAMINE EDESTINATE AND ITS IMMUNE SERUM

Rabbits were given repeated intravenous injections of protamine sulphate, of edestin, or of protamine edestinate, and bled eight days later. The serum was removed by centrifuging, inactivated for one-half hour at 56°C and subsequently used for the fixation experiments.

Injections were made into rabbits as follows: No. 821 was given successive intravenous injections of 10, 20, and 20 milligrams of a solution of protamine sulphate in M/6 NaCl on alternate days and five days later given three more injections of 20 milligrams each on alternate days.

Rabbit 822 was given similar injections of 10 milligrams on three alternate days and after five days given three more injections of 10, 10 and 20 milligrams on alternate days.

Rabbit 817 was given three intravenous injections of 20 milligrams each of edestin dissolved in M/6 NaCl plus several drops of N/10 NaOH, on alternate days and five days later given three more injections of 30, 30 and 50 milligrams on alternate days.

Rabbit 818 was given three similar intravenous injections of 20, 30 and 40 milligrams, each of edestin on alternate days and five days later given three more injections of 40, 50 and 50 milligrams on alternate days.

Rabbit 819 was given three intravenous injections of 20 milligrams each of protamine edestinate dissolved in M/6 NaCl plus several drops of N/10 NaOH on alternate days. The compound protein is much more insoluble than edestin, so that the protamine edestinate was partially in suspension. Five days later 30, 50, and 50 milligrams respectively were injected on alternate days.

Rabbit 820 received three similar injections of 10, 20 and 20 milligrams of the compound protein on alternate days and five days later again injected with 20, 20 and 30 milligrams on alternate days.

Alexin fixation experiments were then carried out with the sera from the above animals and also with the serum of a normal control animal. The criterion for complete fixation was taken as absence of hemolysis after one hour at 37° C. and standing over night in the ice chest.

1/10 c.c. antigen of 1:2500 solution*	821 anti- protamine serum	822 anti- protamine serum	817 anti- edestin serum	818 anti- edestin serum	819 anti- compound serum	820 anti- compound serum	Normal serum
Protamine sulphate	Negative .01 c.c.	Negative .01 c.c.			Negative .01 c.c.	Negative .01 c.c.	Negative .01 c.c.
Edestin			Positive .0012 c.c.	Positive .0016 c.c.	Positive .0012 c.c.	Positive .0012 c.c.	Negative .01 c.c.
Protamine edestinate					Positive .0012 c.c.	Positive .0008 c.c.	Negative .01 c.c.

* This is one-quarter of the minimum inhibiting dose.

Antigens—(a) Protamine sulphate, 1-2500 in M/6 NaCl.

(1/10 c.c.) (b) Edestin, 1-2500 in M/6 NaCl plus slight excess of NaOH.

(c) Protamine edestinate, 1-2500 in M/6 NaCl plus slight excess of NaOH (partial suspension).

Alexin—2/10 c.c. of 5 per cent mixed guinea-pig serum in M/6 NaCl.

Hemolytic system—A 10 per cent suspension of washed sheep corpuscles sensitized with an equal volume of 1-600 hemolysin used in a dosage of 2/10 c.c. This is equivalent to 1/10 c.c. of a 10 per cent suspension sensitized with 1 $\frac{2}{3}$ hemolytic units.

Experiments were also made to determine whether the antibody to protamine edestinate was due to the edestin bound in the compound protein or whether it also gave rise to another antibody for itself. Two cubic centimeters each of serums Nos. 819 and 820 were mixed with an equal volume of a suspension of 25 mg.

edestin in M/6 NaCl. These mixtures were shaken and incubated for one hour at 37° C, then thoroughly centrifuged and the clear serum removed. Alexin fixation experiments were then carried out in a similar manner as previously described, using edestin and protamine edestinate as antigens. The results were negative, even in serum dilutions as low as .02 per cent. The unity of the antibody is apparent. This is in agreement with the work of Gay and Robertson on protamine caseinate.

The substance is non-toxic: a guinea-pig was given 100 milligrams intraperitoneally. No symptoms were shown.

FIXATION EXPERIMENTS WITH GLOBIN-ALBUMOSE COMPOUND

Rabbits were given repeated intravenous injections of globin, of albumose, or of globin-albumose compound, respectively, according to the following protocol, and bled ten days later. The serum was inactivated for one-half hour at 56° C and used for fixation experiments.

Rabbit No. 797 was given intravenous injections of 15 milligrams of acid globin dissolved in M/6 NaCl, for three successive days and five days later three more injections of 20 milligrams each on successive days.

Rabbit 800 was given intravenous injections of 20, 40, 0, 40 milligrams of globin dissolved in M/6 NaCl plus small excess of NaOH, and two days later given three injections of 40 milligrams each on successive days.

Rabbit 796 was given 50 milligrams of deutoalbumose in M/6 NaCl, intravenously, on three successive days and five days later this was repeated.

Rabbit 799 was given intravenous injections of 15, 20 and 20 milligrams each of globin-albumose compound in M/6 NaCl plus small excess of NaOH, on successive days and 5 days later given two injections of 20 milligrams each, on alternate days.

Rabbit 798 was given two injections of 20 milligrams each on successive days and six days later was given three injections of 20 milligrams each on successive days.

Cross-fixation experiments were then carried out in a similar manner as previously described. The antigens were used in a 1-200 dilution as follows:

1/10 c.c. deutoalbumose in M/6 NaCl.

1/10 c.c. globin (two different preparations) in M/6 NaCl plus small excess of NaOH.

i 10 c.c. acid globin (partly neutralized by NaOH) in M/6 NaCl.

l 10 c.c. Globin-albumose compound in M/6 NaCl plus small excess of NaOH.

NOTE. Acid globin when used in too high concentrations will dissolve red corpuscles. In lower dilutions it causes hemagglutination. Partial neutralization overcomes this.

No fixations of alexin were obtained in any of the series, even when dilutions as low as 1 to 10 of serum were used. This confirms the observations of Gay and Robertson and contradicts that of Browning and Wilson,¹³³ who report positive results with globin obtained from the hemoglobin of guinea-pigs. There is no reason for assuming that globin obtained from different species is chemically different, hence it seems probable that the production of antibodies in animals immunized with globin as reported by Browning and Wilson was due to impurities in the preparation. Gay and Robertson were unable to sensitize animals for the anaphylaxis reaction and tests with guinea-pigs show that the substance is toxic.

Globin-albumose compound is toxic on initial injection; the compounding of globin with albumose has apparently not made the compound less toxic than globin. The following experiments show this:

(1) Guinea-pig *B* was given 10 milligrams of a slightly alkaline solution of globin (prepared as in other experiments) intracardially. The animal died in about five minutes.

(2) Guinea-pig *C* was given 2½ milligrams of the same globin preparation intracardially. The animal showed symptoms of partial paralysis but recovered.

(3) Guinea-pig *D* received 10 milligrams of globin-albumose compound (prepared as in previous experiments) intracardially. Death resulted in two minutes.

(4) Guinea-pig *E* was given 5 milligrams of globin-albumose compound as in the above experiment. The animal showed irritation but recovered.

Deuteroalbumose does not give a precipitin reaction with its antiserum, neither does it intoxicate or cause anaphylaxis in a sensitized animal. This is shown in the following experiments:

Rabbit 737 was given three intravenous injections of 100 milligrams each on three successive days and five days later this was repeated. The animal was bled ten days later, the serum inactivated at 56° C, and precipitin tests carried out. The results were negative.

Guinea-pig *A* was given 90 milligrams of deuteroalbumose intraperitoneally and four weeks later another intraperitoneal injection of 200 milligrams. No symptoms were shown.

SUMMARY

1. The literature on the nature of the specificity of such immune reactions as the precipitin, anaphylaxis and alexin fixation has been reviewed.

2. Two general methods have been followed in attacking the problem of the nature of biological specificity: (a) the analytical method, by which workers have attempted either to separate the sensitizing or intoxicating fractions of proteins or sera, or to use pure proteins, closely related biologically, which were found to give group reactions, showing that the specificity is dependent on certain groups in the molecule which may be common to a number of closely related proteins; (b) the synthetic method, whereby compounds of pure protein—antigenic, non-toxic, with non-antigenic, toxic; or non-antigenic, toxic, with non-toxic, non-antigenic are made. Such combinations give a new substance which may be toxic or non-toxic, antigenic or non-antigenic, either for itself or for either or both of the proteins used to build up the compound.

3. Following the latter plan of work, it was found to be necessary to study the reaction which takes place when two proteins unite to form a compound protein.

4. Use of the gas-chain has been made to follow the changes in the H^+ and OH^- concentration in titrating acid and alkaline solutions of globin with solutions of other proteins and of precipitating inorganic salts.

5. Titration curves showing the changes in H^+ and OH^- concentration during titration have been plotted.

6. These titration curves indicate that true protein compounds of globin are formed, some of which may precipitate at a proper acidity or alkalinity while others may be soluble.

7. A distinction can be made by means of the gas-chain between the salting out of globin from solution by inorganic salts and the precipitation of a compound protein.

8. Determination of the H^+ or OH^- concentration in protein solutions used in the preparation of compound proteins serve as a criterion for the duplication of such work.

9. The antigenic properties of two compound proteins—pro-tamine edestinate and globin-albumose compound—have been

studied. It was found that protamine edestinate is non-toxic, and antigenic for itself (due to its edestin content) and edestin, but not for protamine, this being in agreement with the results obtained by Gay and Robertson with protamine caseinate. Globin-albumose compound is non-antigenic, either for itself or for either globin or deuterioalbumose. The compound is toxic.

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REFERENCES

- ¹ Landsteiner, K., Wirken Lipide als Antigene?, Jahresber. Ergebn. Immunitätsforsch., 1910, vol. 6, p. 209.
- ² Fitzgerald, J. G., and Leathes, J. B., The non-antigenic properties of lipoids extracted from human livers with a method of preparation of antigen for use in the Wassermann reaction, Univ. Calif. Publ. Pathol., 1912, vol. 2, p. 39.
- ³ Thiele, F. H., and Embleton, D., On the rôle of lipoids in immunity, Zeitschr. Immunitätsforsch., 1912-3, vol. 16, p. 160.
- ⁴ Ford, W. W.:
 - (a) The toxins and antitoxins of poisonous mushrooms (*Amanita phalloides*), Journ. Infec. Dis., 1906, vol. 3, p. 191.
 - (b) Antibodies to glucosides, with especial reference to *Rhus toxicodendron*, Jour. Infec. Dis., 1907, vol. 4, p. 541.
 - (c) Artificial immunity to glucosides, Science, n.s., 1908, vol. 27, p. 652.
 - (d) Schlesinger, H., and Ford, W. W., On the chemical properties of Amanita-toxin, Jour. Biol. Chem., 1907, vol. 3, p. 279.
 - (e) Rabe, F., Beiträge zur Toxikologie des Knollenblätterschwammes, Zeitschr. exper. Path., 1911, vol. 9, p. 352.
- ⁵ Von Adelung, E., An experimental study of poison oak (*Rhus diversiloba*), Arch. Int. Med., 1913, vol. 11, p. 148.
- ⁶ Portier, P., and Richet, C., De l'action anaphylactique de certains venins, C.-R. Soc. Biol., 1902, Vol. 54, p. 170.
- ⁷ Arthus, M., Injections répétées de serum de cheval chez la lapin, C.-R. Soc. Biol., 1903, vol. 55, p. 817.
- ⁸ Otto, R., Das Theobald Smith'sche Phänomen der Serumüberempfindlichkeit, Von Leuthold Gedenkschrift, 1906, I.
- ⁹ Rosenau, M. J., and Anderson, J. F.:
 - (a) A study of the cause of sudden death following the injection of horse serum, Bull. Hygienic Lab. U. S. P. H. and M. H. S., 1906, no. 29.
 - (b) Further studies upon anaphylaxis, *ibid.*, 1908, no. 45.
 - (c) Further studies upon the phenomenon of anaphylaxis, *ibid.*, 1909, no. 50.
- ¹⁰ Richet, C.:
 - (a) De l'anaphylaxie ou sensibilité croissant des organismes à des doses successives des poison, Arch. Fisiologia, 1903-4, vol. 1, p. 129.
 - (b) De l'action de la congestine sur les lapins et de ses effets anaphylactiques, C.-R. Soc. Biol., 1905, vol. 58, p. 109.
 - (c) Anaphylaxie par la mytilo-congestine, C.-R. Soc. Biol., 1907, vol. 62, p. 358.

¹¹ Von Pirquet, C., and Shack, B., Ueber die Serumkrankheit, Leipzig, 1905.

¹² Biedl, A., and Klaus, R., Experimentelle Studien über Anaphylaxie, Klin. Wochenschr., Wien, 1909, vol. 22, p. 363.

¹³ Friedberger, E., and Hartoch, O., Ueber das Verhalten des Komplements bei der aktiven und passiven Anaphylaxie, Zeitschr. Immunitätsforsch., 1909, vol. 3, p. 581.

¹⁴ Gay, F. P., and Southard, E. E.:

(a) On serum anaphylaxis in the guinea pig, Jour. Med. Res., 1907, vol. 16, p. 143.

(b) On the mechanism of serum anaphylaxis and intoxication in the guinea pig, *ibid.*, 1908, vol. 18, p. 107.

(c) On recurrent anaphylaxis and repeated intoxication in guinea-pigs by means of horse serum, *ibid.*, 1908, vol. 19, p. 1.

(d) The relative specificity in anaphylaxis, *ibid.*, 1908, vol. 19, p. 7.

(e) The localization of cell and tissue anaphylaxis in the guinea-pig with observations on the cause of death in serum intoxication *ibid.*, 1908, vol. 19, p. 17.

Lucas, W. P., and Gay, F. P., Localized anaphylactic intoxication in children following the repeated injection of antitoxin, *ibid.*, 1909, vol. 20, p. 251.

Gay, F. P., Southard, E. E., and Fitzgerald, J. G., Neurophysiological effects of anaphylactic intoxication, *ibid.*, 1909, vol. 21, p. 21.

¹⁵ Nicolle, M., Contribution à l'étude du phénomène d'Arthus, Ann. Inst. Pasteur, 1907, vol. 21, p. 128.

¹⁶ Friedemann, V., Weitere Untersuchungen über den Mechanismus der Anaphylaxie, Zeitschr. Immunitätsforsch., 1909, vol. 2, p. 591.

¹⁷ Pearce, R. M., and Eisenbrey, A. B.:

(a) The physiology of anaphylactic shock in the dog, Jour. Infect. Dis., 1910, vol. 7, p. 565.

(b) A study of the action of the heart in anaphylactic shock in the dog, Jour. Pharm. and Exp. Therap., 1912-3, vol. 4, p. 21.

¹⁸ Auer, J., and Lewis, P. A., The physiology of the immediate reaction of anaphylaxis in the guinea-pig, Jour. Exper. Med., 1910, vol. 12, p. 151.

^{18a} Jobling, J. W., and Peterson, W., The mechanism of anaphylatoxin formation, Jour. Exper. Med., 1914, vol. 20, p. 37.

¹⁹ Richet, C., L'anaphylaxie, Paris, 1912.

²⁰ Gay, F. P., On the relation of anaphylaxis to artificial immunization, Trans. Cong. Amer. Phys. and Surg., 1910, vol. 8, p. 15.

²¹ Rosenau, M. J., and Anderson, J. F., Anaphylaxis, Arch. Int. Med., 1909, vol. 3, p. 519.

²² Doerr, R.:

(a) Allergie und Anaphylaxie, in Kolle and Wassermann, Handbuch der Pathogenen Mikroorganismen, zweite Auflage, Jena, 1913, vol. 2, part 2, p. 947.

(b) Die Anaphylaxie, in Kraus and Levaditi, Handbuch der Technik und Immunitätsforschung, Jena, 1909, vol. 2, p. 856.

^{22a} Zinsser, H., The more recent developments in the study of anaphylactic phenomena, *Arch. Int. Med.*, 1915, vol. 16, p. 223.

²³ Bordet, J.:

(a) Mechanisme de l'agglutination, *Ann. Inst. Pasteur*, 1899, vol. 13, p. 225.

(b) Agglutination et dissolution des globules rouges par le serum, *ibid.*, 1899, vol. 13, p. 273.

Tchistovitch, T., Etudes sur l'immunization contre le serum l'anguilles, *ibid.*, 1899, vol. 13, p. 406.

Myers, W., Ueber Immunität gegen Proteide, *Centralbl. für Bakt.*, 1900, vol. 28, p. 237.

²⁴ Myers, W., On immunity against proteins, *Lancet*, 1900 (2), p. 98.

²⁵ Uhlenhuth, Dr.:

(a) Neuer Beitrag zum spezifischen Nachweis von Eierweiss auf biologischem Wege, *Deutsch. Med. Wochenschr.*, 1900, vol. 26, p. 734.

(b) Zur Lehre von der Unterscheidung verschiedener Eiweissarten mit Hilfe spezifischer Sera, *ibid.*, 1906, p. 1244.

²⁶ Nuttall, G. H. F.:

(a) On the formation of specific antibodies in the blood following upon treatment with the sera of different animals, together with their use in legal medicine, *Jour. Hygiene*, 1901, vol. 1, p. 367.

(b) Progress report upon the biological test for blood as applied to over 500 bloods from various sources, *British Med. Jour.*, 1902 (1), p. 825.

(c) Blood immunity and blood relationship, Cambridge, 1904, p. 205.

²⁷ Graham-Smith, G. S., Blood immunity and blood relationship, Cambridge, 1904, p. 340.

²⁸ Welsh, D. A., and Chapman, H. G., On the differentiation of proteins of closely related species by the precipitin reaction, *Jour. Hygiene*, 1910, vol. 10, p. 177.

²⁹ Levene, P. A., On the biological relationship of proteids, *Medical News*, 1901, vol. 79, p. 981.

³⁰ Umber, F., Zur Chemie und Biologie der Eiweisskörper, *Ber. Klin. Wochenschr.*, 1902, vol. 39, p. 657.

³¹ Uhlenhuth, Dr.:

(a) Eine Methode zur Unterscheidung der verschiedenen Blutarten in besonderem zum differentialdiagnostischen Nachweise des Menschenblutes, *Deutsch. Med. Wochenschr.*, 1901, vol. 27, p. 83.

(b) Weitere Mittheilungen über meine Methode zum Nachweise von Menschenblut, *ibid.*, 1901, vol. 27, p. 260.

(c) Weitere Mittheilungen über die praktische Anwendung meiner forensischen Methode zum Nachweis von Menschen- und Thierblut, *ibid.*, 1901, vol. 27, p. 499.

(d) Die Unterscheidung des Fleisches verschiedener Thiere mit Hilfe spezifischer Sera und die praktische Anwendung der Methode in der Fleischschau, *ibid.*, 1901, vol. 27, p. 780.

³² Wassermann, A., and Schütze, A., Ueber eine neue forensische Methode zur Unterscheidung von Menschen- und Thierblut, Berl. Klin. Wochenschr., 1901, vol. 38, p. 187.

Ewing, J., and Strauss, I., Limits of a specific reaction in the serum test for blood, Proc. N. Y. Path. Soc., 1902-3, vol. 2, p. 152.

³⁴ Ewing, J., Differentiation of monkey and human blood by the serum test, Proc. N. Y. Path. Soc., 1903-04, vol. 3, p. 14.

Stern, R., Ueber den Nachweis menschlichen Blutes durch ein Antiserum, Deutsch. Med. Wochenschr., 1901, vol. 27, p. 135.

³⁶ Wassermann, A., Ueber neue Versuche auf dem Gebiete der Serumtherapie, Cong. f. inn. Med., 1900, p. 566.

³⁷ Grünbaum, A. S. F., Note on the blood relationship of man and the anthropoid apes, Lancet, 1902 (1), p. 143.

³⁸ Strube, G., Beiträge zum Nachweis von Blut und Eiweiss auf biologischen Wege, Deutsch. Med. Wochenschr., 1902, vol. 28, p. 425.

³⁹ Linossier, G., et Lemoine, G. H., Sur les substances précipitantes des albumines (précipitines) contenues dans certains sérums spécifiques, C.-R. Soc. Biol., 1902, vol. 54, p. 85.

⁴⁰ Gay, F. P., A contribution to the forensic value of the musculo-precipitin test, Jour. Med. Res., 1908, vol. 19, p. 219.

⁴¹ Rosenau, M. J., and Anderson, J. F.:

(a) A study of the cause of sudden death following the injection of horse serum, Bull. Hygienic Lab. U. S. P. H. and M. H. S., 1906, no. 29.

(b) Further studies upon the phenomenon of anaphylaxis, *ibid.*, 1909, no. 50.

(c) The specific nature of anaphylaxis, Jour. Infec. Dis., 1907, vol. 4, p. 552.

(d) A review of anaphylaxis, with especial reference to immunity, *ibid.*, 1908, vol. 5, p. 85.

(e) Further studies upon anaphylaxis, Jour. Med. Res., 1908, vol. 19, p. 37.

⁴² Thomsen, O.:

(a) Ueber die Spezifität der Serumanaphylaxie und die Möglichkeit ihrer Anwendung in der medikoforensischen Praxis zur Differenzierung von Menschen- und Tierblut, Zeitschr. Immunitätsforsch., 1909, vol. 1, p. 741.

(b) Untersuchungen über die Blutanaphylaxie und die Möglichkeit ihrer Anwendung in der Gerichtsmedizin, *ibid.*, 1909, vol. 3, p. 539.

⁴³ Kapsenberg, G., Die Anaphylaxie mit Linsensubstanz, Zeitschr. Immunitätsforsch., 1912, vol. 15, p. 518.

⁴⁴ Wells, H. G., Studies on the chemistry of anaphylaxis, Jour. Infec. Dis., 1908, vol. 5, p. 449.

⁴⁵ Besredka, Dr., De l'anaphylaxie lactique, Ann. Inst. Past., 1909, vol. 23, p. 166.

⁴⁵ Uhlenhuth, Dr., and Haendel, Dr., Untersuchungen über die praktische Verwertbarkeit der Anaphylaxie zur Erkennung und Unterscheidung verschiedener Eiweisarten, *Zeitschr. Immunitätsforsch.*, 1910, vol. 4, p. 761.

⁴⁶ Kraus, R., Doerr, R., and Schma, Dr., Ueber Anaphylaxie hervorgerufen durch Organextrakte (Linsen), *Wien Klin. Wochenschr.*, 1908, vol. 21, p. 1984.

⁴⁸ Andrejew, P., Ueber Anaphylaxie mit Eiweiss tierischer Linsen, *Arbeit. Kais. Gesundheitsamte*, 1909, vol. 30, p. 450.

⁴⁹ Pfeiffer, H., and Mita, S., Studien über Eiweiss-Anaphylaxie, *Zeitschr. Immunitätsforsch.*, 1910, vol. 4, p. 410.

⁵⁰ Friedberger, E., and Goretta, G., Bewirkt arteigenes "blutfremdes" Eiweiss bei wiederholter Zufuhr, Ueberempfindlichkeit?, *Zeitschr. Immunitätsforsch.*, 1914, vol. 21, p. 668.

⁵¹ Krusius, F. F., Zur biologischen Sonderstellung der Linse, *Zeitschr. Immunitätsforsch.*, 1910, vol. 5, p. 699.

⁵² Bordet, J., and Gengou, O., Sur l'existence de substances sensibilisatrices dans la pleupart des sérums antimicrobiens, *Ann. Inst. Past.*, 1901, vol. 15, p. 289.

⁵³ Gengou, O., Sur les sensibilisatrices des sérums actifs contre les substances albuminoïdes, *Ann. Inst. Past.*, 1902, vol. 16, p. 734.

⁵⁴ Ballner, F., Ueber die Differenzierung von pflanzlichem Eiweiss mittels der Komplementbildungsreaktion, *S.-B. Kais. Akad. Wissensch. Wien, Math.-Naturw. Kl.*, 1910, vol. 119, p. 17.

⁵⁵ Dunbar, W. P., Ueber das serobiologische Verhalten der Geschlechtszellen, *Zeitschr. Immunitätsforsch.*, 1910, vol. 4, p. 740.

⁵⁶ Wendelstadt, Prof. and Fellner, T., Beitrag zur Kenntnis der Immunisierung durch Pflanzeneiweiss, *Zeitschr. Immunitätsforsch.*, 1910-11, vol. 8, p. 43.

⁵⁷ Bauer, J., Ueber die biologische Differenzierung von Körperflüssigkeiten derselben Thierart, *Zeitschr. Exper. Path. und Therap.*, 1909-10, vol. 7, p. 417.

⁵⁸ Brück, C., Die biologische Differenzierung von Affenarten und menschlichen Rassen durch spezifische Blutreaction, *Berl. Klin. Wochenschr.*, 1907, vol. 44, p. 793.

⁵⁹ Fitzgerald, J. G., An attempt to show specific racial differences in human blood by means of the reaction of fixation, *Jour. Med. Res.*, 1909, vol. 21, p. 41.

⁶⁰ Hardy, W. B., Colloidal solution: the globulins, *Jour. Physiol.*, 1905-6, vol. 33, p. 251.

⁶¹ Fleischer, G. W., Zur Biologie der Präzipitine (Russky Wratsch., 1908, no. 49), via *Centralbl. allgem. Path. u. path. Anat.*, 1909, vol. 20, p. 308.

⁶² Wells, H. G.:

(a) Studies on the chemistry of anaphylaxis, *Jour. Infec. Dis.*, 1908, vol. 5, p. 449.

(b) Studies on the chemistry of anaphylaxis, II, *ibid.*, 1909, vol. 6, p. 506.

⁶³ Wells, H. G., Studies on the chemistry of anaphylaxis, III: Experiments with isolated proteins, especially those of the hen's egg, *Jour. Infect. Dis.*, 1911, vol. 9, p. 117.

⁶⁴ Taylor, A. E., Chemical studies in cytotoxicity, *Jour. Biol. Chem.*, 1908-9, vol. 5, p. 311.

⁶⁵ Wells, H. G., and Osborne, T. B.:

(a) The biological reactions of the vegetable proteins, *Jour. Infect. Dis.*, 1911, vol. 8, p. 66.

(b) Is the specificity of the anaphylaxis reaction dependent on the chemical constitution of the proteins or on their biological relations?, *ibid.*, 1913, vol. 12, p. 341.

(c) The anaphylactogenic activity of some vegetable proteins, *ibid.*, 1914, vol. 14, p. 377.

(d) The anaphylactic reaction with so-called proteoses of various seeds, *ibid.*, 1915, vol. 17, p. 259.

(e) Anaphylaxis reactions between proteins from seeds of different genera of plants, *ibid.*, 1916, vol. 19, p. 183.

Lake, G. C., Osborne, T. B., and Wells, H. G., The immunological relationship of hordein of barley and gliadin of wheat as shown by the complement fixation, passive anaphylaxis, and precipitin reactions, *ibid.*, 1914, vol. 14, p. 364.

⁶⁶ White, B., and Avery, O. T., Some immunity reactions of edestin, *Jour. Infect. Dis.*, 1913, vol. 13, p. 103.

⁶⁷ Elliott, C. H., The antigenic properties of glycoproteins, *Jour. Infect. Dis.*, 1914, vol. 15, p. 501.

⁶⁸ Obermeyer, F., and Pick, E. P., Ueber die chemischen Grundlagen der Art-Eigenschaften der Eiweisskörper, *Wien. Klin. Wochenschr.*, 1906, vol. 19, p. 327.

⁶⁹ Landsteiner, K., and Jablons, B., Ueber die Antigeneigenschaften von acetyliertem Eiweiss, *Zeitschr. Immunitätsforsch.*, 1914, vol. 21, p. 193.

⁷⁰ Pick, E. P., and Yamanouchi, T., Chemische und experimentelle Beiträge zum Studium der Anaphylaxie, *Zeitschr. Immunitätsforsch.*, 1909, vol. 1, p. 676.

⁷¹ See also Thompson, W. H., Die physiologische Wirkung der Protamine und ihrer Spaltungsprodukte, *Zeitschr. physiol. Chem.*, 1900, vol. 29, p. 1.

McCruden, F. H., The toxic action of certain fish ovaries, *Amer. Chem. Soc. Abst.*, 1912, vol. 6, p. 1181.

Gay, F. P. and Robertson, T. B., The antigenic properties of a protein compounded with casein, *Jour. Exper. Med.*, 1912, vol. 16, p. 479.

⁷² Persano, E., Action de la peptone dans le sang du cobaye et du crapaud, *Archiv. Ital. de Biol.*, 1902, vol. 37, p. 409.

Botazzi, F., Contribution à la connaissance de la coagulation du sang de quelques animaux marins et des moyens pour l'empêcher, *ibid.*, 1902, vol. 37, p. 409.

Thompson, W. H., Contribution to the physiological effects of peptone when injected into the circulation, *Jour. Physiol.*, 1896, vol. 20, p. 455.

⁷³ Nolf, Bull. Acad. Royal Belgique, 1902, no. II, p. 866, cited after Underhill, F. P., *Amer. Jour. Physiol.*, 1903, vol. 9, p. 345.

⁷⁴ Underhill, F. P., New experiments on the physiological action of the proteoses, *Amer. Jour. Physiol.*, 1903, vol. 9, p. 345.

Underhill, F. P., and Hendrix, B. M.:

(a) Studies on the physiological action of some protein derivatives: I. Are proteoses prepared from zein and gliadin physiologically active?, *Jour. Biol. Chem.*, 1915, vol. 22, p. 443.

(b) The relation of racemization to the physiological action of proteins and proteoses, *ibid.*, p. 453.

(c) The physiological action of Vaughan's crude soluble poison, *ibid.*, p. 465.

⁷⁵ Richet, C., Rôle du système dans les phénomènes de l'anaphylaxie aigue, *Presse Med.*, 1909, vol. 17, p. 249.

⁷⁶ Zunz, E., Recherches sur l'anaphylaxie par le protéoses, *Zeitschr. Immunitätsforsch.*, 1912-13, vol. 16, p. 580.

Zunz, E., and György, P., A propos de la toxicité des protéoses et de l'anaphylaxie par ces composés, *ibid.*, 1914, vol. 23, p. 296.

⁷⁷ Gay, F. P., and Robertson, T. B., The antigenic properties of split products of casein, *Jour. Exper. Med.*, 1912, vol. 16, p. 470.

⁷⁸ Schittenhelm, A., and Weichardt, W.:

(a) Studien über die biologische Wirkung bestimmter parenteral einverleibter Eiweisspaltprodukte, *Zeitschr. Immunitätsforsch.*, 1912, vol. 14, p. 609.

(b) Ueber die biologische Differencirung von Eiweiss- und Eiweisspaltproducten durch ihre Wirkung auf den thierischen Organismus, *Zeitschr. exper. Path.*, 1912, vol. 11, p. 69.

Schittenhelm, A., and Ströbel, H., Ueber die Giftigkeit arteigener Eiweissabbauprodukte, *Zeitschr. exper. Path.*, 1912, vol. 11, p. 108.

⁷⁹ Bürger, M., Studien über die praktische Verwertbarkeit der Anaphylaxie bei Sensibilisierung mit denaturiertem Eiweiss, *Zeitschr. Immunitätsforsch.*, 1914, vol. 22, p. 199.

⁸⁰ Robertson, T. B.:

(a) Note on the synthesis of a protein through the action of pepsin, *Jour. Biol. Chem.*, 1907, vol. 3, p. 95.

(b) On the synthesis of paranuclein through the agency of pepsin and the chemical mechanics of the hydrolysis and synthesis of proteins through the agency of enzymes, *ibid.*, 1908-9, vol. 5, p. 393.

(c) On the refractive indices of solutions of certain proteins: the paranucleins, *ibid.*, 1910, vol. 8, p. 287.

Robertson, T. B., and Biddle, H. C., On the composition of certain substances produced by the action of pepsin upon the products of the complete peptic hydrolysis of casein, *ibid.*, 1911, vol. 9, p. 295.

⁸¹ Vaughan, V. C., and Wheeler, S. M., The effects of egg white and its split products; a study of susceptibility and immunity, *Jour. Infect. Dis.*, 1907, vol. 4, p. 476.

Leach, M. F.:

(a) On the chemistry of bacillus coli communis: the non-poisonous portion, *Jour. Biol. Chem.*, 1905-6, vol. 1, p. 463, and 1907, vol. 3, p. 443.

(b) A preliminary study of the sensitizing portion of egg-white, *ibid.*, 1908-9, vol. 5, p. 253.

Edmunds, C. W., The action of the protein poison on dogs: a study in anaphylaxis, *Zeitschr. Immunitätsforsch.*, 1913, vol. 17, p. 105.

Vaughan, V. C., Protein split products in relation to immunity and disease, Philadelphia, 1913.

⁸² Gay, F. P., and Adler, H. M., On the chemical separation of the sensitizing fraction (anaphylactin) from horse serum, *Jour. Med. Res.*, 1908, vol. 18, p. 433.

⁸³ Doerr, R., and Russ, V. K., Die Identität der anaphylaktisierenden und der toxischen Substanz artfremder Sera, *Zeitschr. Immunitätsforsch.*, 1909, vol. 2, p. 109.

⁸⁴ Braun, H., Zur Frage der Serumempfindlichkeit, *Zeitschr. Immunitätsforsch.*, 1910, vol. 4, p. 590.

⁸⁵ Hall, I. C., Studies on conglutination, *Univ. Calif. Publ. Pathol.*, 1913, vol. 2, p. 111.

⁸⁶ Armit, H. W., Hypersensitivity to pure egg-albumin, *Zeitschr. Immunitätsforsch.*, 1910, vol. 6, p. 703.

⁸⁷ Robertson, T. B.:

(a) Die physikalische Chemie der Proteine, Dresden, 1912, p. 126.

(b) The proteins, *Univ. Calif. Publ. Physiol.*, 1911, vol. 4, p. 25.

(c) The preparation and properties of a compound protein: globin caseinate, *Jour. Biol. Chem.*, 1912-3, vol. 13, p. 499.

⁸⁸ Robertson, T. B.:

(a) The proteins of ox-serum; a new optical method of determining the concentrations of the various proteins contained in blood-sera, *Jour. Biol. Chem.*, 1912, vol. 11, p. 179.

(b) A comparison of the sera of the horse, rabbit, rat, and ox with respect to their content of various proteins in the normal and in the fasting condition, *ibid.*, 1912-3, 13, p. 325.

Wooley, J. H., A comparison of the sera of the ox, sheep, hog, goat, dog, cat, and guinea-pig with respect to their content of various proteins, *ibid.*, 1913, vol. 14, p. 433.

Wells, C. E., The influence of age and of diet on the relative proportions of serum proteins in rabbits, *ibid.*, 1913, vol. 15, p. 37.

Thompson, W. B., A comparison of the sera of the hen, turkey, duck, and goose, with respect to their content of various proteins, *ibid.*, 1915, vol. 20, p. 1.

Briggs, R. S., A comparison of the sera of the pigeon, rooster, and guinea-fowl with respect to their content of various proteins in the normal and in the fasting condition, *ibid.*, 1915, vol. 20, p. 7.

Buck, L. W., Effects of chloroform and of ether anesthesia on the protein contents of the blood-serum of rabbits, *Jour. Pharm. and Exper. Therap.*, 1913-4, vol. 5, p. 553.

⁸⁹ Reichert, E. T., and Brown, A. P., The differentiation and specificity of corresponding proteins and other vital substances in relation to biological classification and organic evolution. The crystallography of hemoglobins, Carnegie Inst. Publ., 1909, no. 116.

Reichert, E. T.:

(a) The differentiation and specificity of starches in relation to genera, species, etc., Carnegie Inst. Publ., 1913, no. 173.

(b) The germplasm as a stereochemic system, *Science*, n.s., 1914, Nov. 6, p. 649.

For reactions with hemoglobin see

Ford, W. W., and Halsey, J. T., Contributions to the study of hemagglutinins and hemolysins, *Jour. Med. Res.*, 1904, vol. 11, p. 403.

Bradley, H. C., and Sansum, W. D., Some anaphylactic reactions, *Jour. Biol. Chem.*, 1914, vol. 18, p. 497.

⁹⁰ Beebe, S. P.:

(a) Cytotoxic serum produced by the injection of nucleoproteids, *Jour. Exper. Med.*, 1905, vol. 7, p. 733.

(b) Artificial immunization in non-bacterial diseases, *Jour. Am. Med. Assn.*, 1910, vol. 55, p. 1712.

Guerrini, G., Beitrag zum Studium der Anaphylaxie: Ueber Anaphylaxie durch Gewebe- und Bakterienproteide, *Zeitschr. Immunitätsforsch.*, 1912, vol. 14, p. 70.

⁹¹ Pearce, R. M., and Jackson, H. C., Concerning the production of cytotoxic sera by the injection of nucleoproteids, *Jour. Infect. Dis.*, 1906, vol. 3, p. 742.

Pearce, R. M., Karsner, H. T., and Eisenbrey, A. B., Studies in immunity and anaphylaxis: the proteins of the kidney and liver, *Jour. Exper. Med.*, 1911, vol. 14, p. 44.

Lake, G. C., The immunological reactions of the proteins of the human placenta with especial reference to the production of a therapeutic serum for malignant chlorin-epithelioma, *Jour. Infect. Dis.*, 1914, vol. 14, p. 385.

Taylor, A. E., Chemical studies in cytotoxicity, *Jour. Biol. Chem.*, 1908-9, vol. 5, p. 311.

Abderhalden, E., and Kashiwado, T., Studien über die Kerne der Thy-musdrüse und Anaphylaxieversuche mit Kernsubstanzen (Nucleoprotein, Nuclein, und Nucleinsäuren), *Zeitschr. physiol. Chem.*, 1912, vol. 81, p. 285.

Wells, H. G., Nucleo-proteins as antigens, *Zeitschr. Immunitätsforsch.*, 1913, vol. 19, p. 599.

⁹² Gay, F. P., and Robertson, T. B.:

(a) The antigenic properties of a protein compounded with casein, *Jour. Exper. Med.*, 1912, vol. 16, p. 479.

(b) The antigenic properties of globin caseinate, *ibid.*, 1913, vol. 17, p. 535.

⁹³ Kossel, A., Ueber Lymphzellen, *Deutsch. med. Wochenschr.*, 1894, vol. 20, p. 146.

⁹⁰ Kutscher, F., Zur Kenntniss der ersten Verknüpfungsstadien des Eiweiss, *Zeitschr. physiol. Chem.*, 1897, vol. 23, p. 115.

⁹¹ Bang, L., Studien über Histon, *Zeitschr. physiol. Chem.*, 1899, vol. 27, p. 463.

⁹² Kossel, A., and Kutscher, F., Beiträge zur Kenntniss der Eiweisskörper, *Zeitschr. physiol. Chem.*, 1900-1, vol. 31, p. 165.

⁹³ Kossel, A., and Pringle, H., Ueber Protamine und Histare, *Zeitschr. physiol. Chem.*, 1906, vol. 49, p. 301.

⁹⁴ Hunter, A., Ueber die Verbindungen der Protamine mit anderen Eiweisskörpern, *Zeitschr. physiol. Chem.*, 1907, vol. 53, p. 526.

⁹⁵ Altmann, R., Ueber Nucleinsäuren, *Archiv. Anat. u. Physiol. (Physiol. Abt.)*, 1889, p. 524.

¹⁰⁰ Milroy, T. H., Ueber die Eiweiss-Verbindungen der Nucleinsäure und Thyminsäure und ihre Beziehung zu den Nucleinen und Paracucleinen, *Zeitschr. physiol. Chem.*, 1896-7, vol. 22, p. 307.

¹⁰¹ Böttger, W., Die Anwendung des Elektrometers als Indikator beim Titrieren von Säuren und Basen, *Zeitschr. physik. Chem.*, 1897, vol. 24, p. 253.

¹⁰² Hildebrand, J. H., Some applications of the hydrogen electrode in analysis, research and teaching, *Jour. Am. Chem. Soc.*, 1913, vol. 35, p. 847.

¹⁰³ Salm, E.:

(a) Die Bestimmung des H-Gehaltes einer Lösung mit Hilfe von Indikatoren, *Zeitschr. Electrochem.*, 1904, vol. 10, p. 341.

(b) Studie über Indikatoren, *Zeitschr. physik. Chem.*, 1906-7, vol. 57, p. 471.

¹⁰⁴ Schmidt, C. L. A., and Finger, C. P., Potential of a hydrogen electrode in acid and alkaline borate solutions, *Jour. Phys. Chem.*, 1908, vol. 12, p. 406.

¹⁰⁶ Hildebrand, J. H., and Bowers, W. G., A study of the action of alkali on certain zinc salts by means of the hydrogen electrode, *Jour. Am. Chem. Soc.*, 1916, vol. 38, p. 785.

¹⁰⁵ Noyes, A. A., and Whitney, W. R., Untersuchungen mit Aluminaten und Boraten von Alkalimetallen, *Zeitschr. phys. Chem.*, 1894, vol. 15, p. 694.

¹⁰⁶ Kahlenberg, L., and Schreiner, O., Ueber Borsäure und ihre Salze, *Zeitschr. phys. Chem.*, 1896, vol. 20, p. 547.

¹⁰⁷ Shelton, H. S., Ueber den Molekularzustand des Borax in Lösung, *Zeitschr. phys. Chem.*, 1903, vol. 43, p. 494.

¹⁰⁸ Tammann, G.:

(a) Ueber die Ermittlung der Zusammensetzung chemischer Verbindungen ohne Hilfe der Analyse, *Zeitschr. anorg. Chem.*, 1903, vol. 37, p. 303.

(b) Ueber die Anwendung der thermischen Analyse in abnormen Fällen, *ibid.*, 1905, vol. 45, p. 24.

(c) Ueber die Anwendung der thermischen Analyse (3), *ibid.*, 1905, vol. 47, p. 289.

Schmidt, C. L. A., Methods in obtaining cooling curves, *Calif. Jour. Tech.*, 1907, vol. 9, p. 15.

Schmidt, C. L. A., and Watkins, W. K., The thermal analysis of alloys, *Calif. Jour. of Tech.*, 1908, vol. 11, p. 7.

¹⁰⁹ Robertson, T. B., On the dissociation of serum globulin at varying hydrogen ion concentrations, *Jour. Phys. Chem.*, 1907, vol. 11, p. 437.

¹¹⁰ Robertson, T. B., The dissociation of potassium caseinate in solutions of varying alkalinity, *Jour. Phys. Chem.*, 1910, vol. 14, p. 528.

¹¹¹ Robertson, T. B., The dissociation of the salts of ovomucoid in solutions of varying alkalinity and acidity, *Jour. Phys. Chem.*, 1910, vol. 14, p. 709.

¹¹² Robertson, T. B., and Schmidt, C. L. A., On the part played by the alkali in the hydrolysis of proteins by trypsin, *Jour. Biol. Chem.*, 1908, vol. 5, p. 31.

¹¹³ Michaelis, L., *Die Wasserstoffionenkonzentration, ihre Bedeutung für die Biologie und die Methoden ihrer Messung*, Berlin, 1914.

^{113a} Sørensen, S. P. L., Ueber die Messung und Bedeutung der Wasserstoffionenkonzentration bei biologischen Prozessen, *Ergeb. der Physiol.*, 1912, vol. 12, p. 393.

¹¹⁴ Robertson, T. B., *Die physikalische Chemie der Proteine*, Dresden, 1912, p. 417.

¹¹⁵ Robertson, T. B., On the dissociation of serum globulin at varying hydrogen ion concentrations, *Jour. Phys. Chem.*, 1907, vol. 11, p. 442.

Schmidt, C. L. A., and Finger, C. P., Potential of a hydrogen electrode in acid and alkaline borate solutions, *Jour. Phys. Chem.*, 1908, vol. 12, p. 406.

Loomis, N. E., A study of the hydrogen electrode, of the calomel electrode, and of contact potential, *Dissertation Johns Hopkins University*, 1911, p. 22.

Loomis, N. E., and Acree, S. F., A study of the hydrogen electrode, of the calomel electrode, and of contact potential, *Am. Chem. Jour.*, 1911, vol. 46, p. 602.

¹¹⁶ Richards, T. W., Ueber den Temperaturekoeffizienten des Potentials der Kalomelelektrode mit verschiedenen gelösten Elektrolyten, *Zeitschr. phys. Chem.*, 1897, vol. 24, p. 39.

¹¹⁷ Bjerrum, N., Ueber die Elimination des Diffusionspotentials zwischen verdünnten wässrigen Lösungen durch Einschalten einer konzentrierten Chlorkaliumlösung, *Zeitschr. phys. Chem.*, 1905, vol. 53, p. 428.

Loomis, N. E., and Acree, S. F., A study of the hydrogen electrode, of the calomel electrode, and of contact potential, *Am. Chem. Jour.*, 1911, vol. 46, p. 602.

Clark, F. W., Myers, C. N., and Acree, S. F., A study of the hydrogen electrode, of the calomel electrode, and of contact potential, *Jour. Phys. Chem.*, 1916, vol. 20, p. 241.

See also—

Abegg, R., and Cummings, A. C., Zur Eliminierung der Flüssigkeitspotentiale, *Zeitschr. elekt. Chem.*, 1907, vol. 13, p. 17.

¹¹⁸ Desha, L. J., and Acree, S. F., On the difficulties in the use of the hydrogen electrode in the measurement of the concentration of hydrogen ions in the presence of organic compounds, *Am. Chem. Jour.*, 1911, vol. 6, p. 638.

¹¹⁹ Schmidt, C. L. A., Table of H^+ and OH^- concentrations corresponding to electromotive forces determined in gas-chain measurements, *Univ. Calif. Publ. Physiol.*, 1909, vol. 3, p. 101.

¹²⁰ Robertson, T. B., On the refractive indices of solutions of certain proteins: globin, *Jour. Biol. Chem.*, 1913, vol. 13, p. 455.

¹²¹ Robertson, T. B.:

(a) Studies in the chemistry of ion-protein compounds, *Jour. Biol. Chem.*, 1907, vol. 2, p. 317.

(b) The dissociation of potassium caseinate in solutions of varying alkalinity, *Jour. Phys. Chem.*, 1910, vol. 14, p. 534.

¹²² Plattner, E. A., Ueber die Darstellung und die Bestandtheile der in der Galle enthaltenen krystalisirbaren Natronverbindung, *Jour. prakt. Chem.*, 1847, vol. 40, p. 129.

¹²³ Pauli, W., Hofmeisters Beiträge, 1906, vol. 7, p. 531, cited after Robertson, T. B., *Die physikalische Chemie der Proteine* (Dresden, 1912), p. 84.

¹²⁴ Hofmeister, F.:

(a) Zur Lehre von der Wirkung der Salze, *Archiv. exper. Path.*, 1888, vol. 24, p. 247; (b) 1889, vol. 25, p. 1; (c) 1890, vol. 27, p. 395; (d) 1891, vol. 28, p. 210.

¹²⁵ Galeotti, G.:

(a) Ueber die sogenannten Metallverbindungen der Eiweisskörper nach der Theorie der chemischen Gleichgewichte, *Zeitschr. physiol. Chem.*, 1903-4, vol. 40, p. 492.

(b) Ueber die Konzentration der Metallionen in eiweisshaltigen Silbernitratlösungen, *ibid.*, 1904, vol. 42, p. 330.

¹²⁶ Schulz, F. N., Der Eiweisskörper des Hämoglobins, *Zeitschr. physiol. Chem.*, 1898, vol. 24, p. 449.

¹²⁷ Kossel, A., Ueber die Constitution der einfachen Eiweissstoffe, *Zeitschr. physiol. Chem.*, 1898, vol. 25, p. 165.

¹²⁸ Taylor, A. E.:

(a) On the hydrolysis of protamine with especial reference to the action of trypsin, *Univ. Calif. Publ. Pathol.*, 1904, vol. 1, p. 7.

(b) On the synthesis of protein through the action of trypsin, *ibid.*, 1907, vol. 1, p. 343.

(c) On the synthesis of protein through the action of trypsin, *Jour. Biol. Chem.*, 1907, vol. 3, p. 87.

¹²⁹ Robertson, T. B.:

(a) Refractive indices of solutions of certain proteins: salmin, *Jour. Biol. Chem.*, 1912, vol. 11, p. 307.

(b) On the rate of extraction of a protein (salmin) from dessicated tissue by an aqueous solvent, *ibid.*, 1913, vol. 14, p. 237.

Robertson, T. B., On the part played by the alkali in the hydrolysis of proteins by trypsin, *Jour. Biol. Chem.*, 1908, vol. 5, p. 31.

Gay, F. P., and Robertson, T. B., The antigenic properties of a protein compounded with casein, *Jour. Exper. Med.*, 1912, vol. 16, p. 479.

¹³⁰ Osborne, T. B.:

(a) Crystallized proteid of hempseed, *Am. Chem. Jour.*, 1892, vol. 14, p. 671.

(b) The basic character of the protein molecule and the reactions of edestin with definite quantities of acids and alkalies, *Jour. Am. Chem. Soc.*, 1902, vol. 24, p. 39.

Chittenden, R. H., and Mendel, L. B., On the proteolysis of crystallized globulin, *Jour. Physiol.*, 1894-5, vol. 17, p. 48.

¹³¹ Schmidt, C. L. A., A large fat extractor, *Jour. Indust. and Engin. Chem.*, 1916, vol. 8, p. 165.

¹³² Schmidt, C. L. A., On the refractive indices of solutions of certain proteins: edestin, *Jour. Biol. Chem.*, 1915, vol. 23, p. 487.

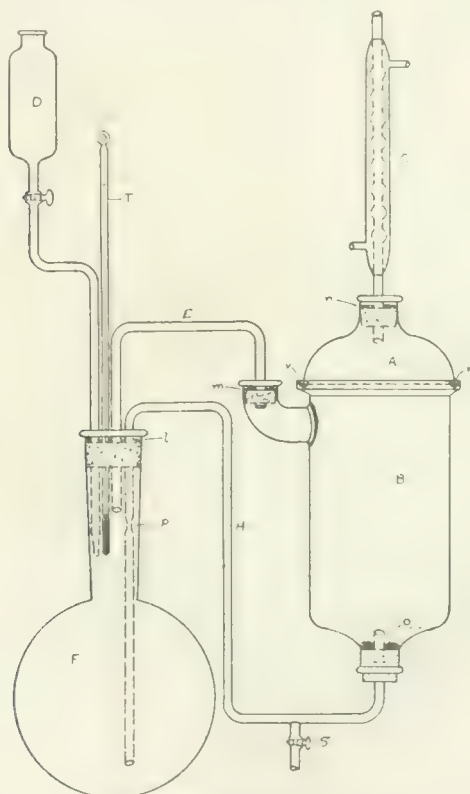
¹³³ Browning, C. H., and Wilson, G. H., An anti-substance to globin and its properties, *Jour. Path. and Bact.*, 1909, vol. 14, p. 174.

A LARGE FAT EXTRACTOR

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Nearly all the types of fat extractors which have been described have been designed for the purpose of quantitatively determining fat in some particular substance rather than merely freeing a substance of its fat content so that the fat-free residuum may be used. The quantitative determination of fat can usually be made on a comparatively small sample and for such purposes the various types of fat extrac-



FAT EXTRACTING APPARATUS 1/3 ACTUAL SIZE

tors on the market are well adapted. But when the fat from several pounds of material must be extracted, as is generally the case in the preparation of vegetable proteins, in order to obtain even a comparatively

small yield of protein, recourse is generally had to some home-made type of fat extraction apparatus, since there are no extractors on the market adapted for this particular purpose.

Benson and Thompson¹ have described an extractor used by them in extracting tannin from sawdust. A somewhat similar apparatus was used by C. H. McCharles, of this University, for the extraction of fat from vegetable substances used in the preparation of pure proteins. I have made an extractor similar in principle by using a filtering bell jar and the top of a vacuum desiccator, both having ground glass joints. But a ground glass joint, even when clamped, will not prevent leakage of ether vapor. The apparatus described below has mercury seals at all connections, thus preventing any leakage of ether vapor. In addition to its use in extracting fat from several pounds of material, the apparatus may be used in the recovery of the ether used in the extraction and it is even adapted for the distillation of anhydrous ether.

The extraction apparatus consists essentially of two parts, a large glass distilling flask, *F*, and the extractor *AB*, the latter made of heavy glass. The upper part, *A*, of the extractor, fits into a groove, *V*, which can be filled with mercury, thus insuring an ether-tight seal. The other joints, *l*, *m*, *n*, *o*, are likewise made ether-tight by means of mercury seals. The material to be extracted is put in a large, hardened filter paper shaped into a bag and placed in *B*, the bottom of the bag resting on a small porcelain filter plate. Vapor from the boiling ether in *F* will pass through the tube *E* and be condensed at *A*, dripping on the substance in *B*. When the ether in *B* has reached the height of the tube *H* it will siphon back into the flask *F*, provided a small constriction be made in the glass tube at *P*. The ether used in the extraction may be recovered by distilling into *B* and draining through the stopcock *S*. It may be of advantage to insert a glass siphon into the flask *F* which will eliminate the trouble of disconnecting the flask to remove the fatty residuum. Action of ether on the corks *l*, *m*, *n* may be prevented by coating them with gelatine or glue.

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Benson and Thompson, THIS JOURNAL, 7 (1915), 916.

AN ELECTRICALLY HEATED VACUUM DESICCATOR.*

By T. BRAILSFORD ROBERTSON AND CARL L. A. SCHMIDT

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In preparing certain biochemical products it is frequently desirable to employ a desiccator of large capacity, capable of evacuation and of being heated and maintained at a predetermined temperature. The various forms of apparatus available on the market are either of very small capacity or else expensive and deficient in the range and adjustability of the temperatures employed. We accordingly have had constructed for our use the apparatus described below, which is of large capacity, readily accommodating a filter funnel 9 inches in diameter containing a 50 cm. filter paper. It maintains a vacuum for several hours and may be held with considerable precision at any desired temperature within a wide range. If desired, a vessel containing sulfuric acid or other desiccating reagent may be placed on the floor of the apparatus and false bottoms or shelves may be fixed at any desired height above the reagent. With the aid of this apparatus, employing certain obvious precautions, tethelin suspended in alcohol-ether mixture may be completely dried without discoloration at 36°C. within 6 hours, yet this substance is so exceedingly hygroscopic that a few minutes' exposure to the air of the room suffices to convert it into a deeply colored, sticky mass.¹

The apparatus consists of a double walled circular copper ($\frac{1}{16}$ inch sheet copper) chamber, well insulated by asbestos and set in a monel metal shell. This chamber can be closed at the top

* Aided in part by a grant from the George Williams Hooper Foundation for Medical Research.

¹ Robertson, T. B., *J. Biol. Chem.*, 1916, xxiv, 409.

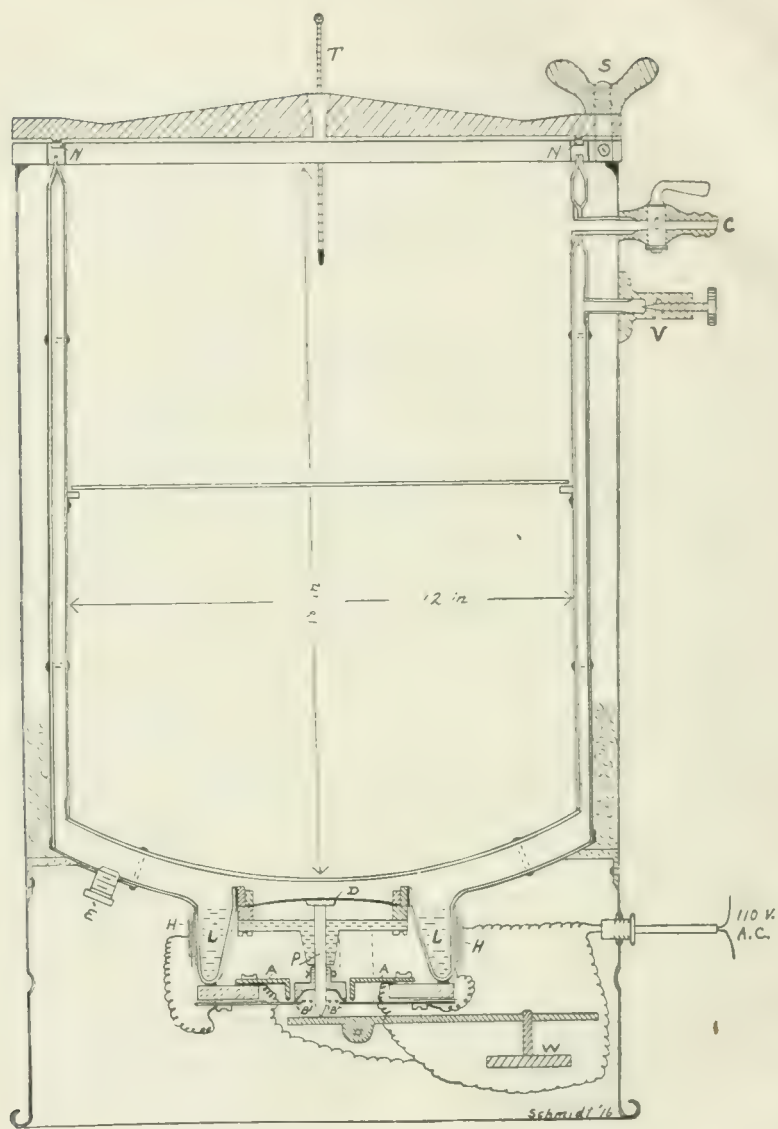


FIG. 1. An electrically heated vacuum desiccator. Scale $\frac{1}{2}$.

by a tight fitting cast aluminum lid which can be screwed down by means of three screws (S). A rubber gasket in the notched space (N) makes the compartment air-tight. The chamber is evacuated by attaching a vacuum pump to the cock (C) and the vacuum so obtained can readily be maintained for some time after the cock is closed. The desired temperature in the evacuated chamber is maintained by the heated vapor of dichloromethane (boiling point $-40^{\circ}\text{C}.$) which fills the space between the two copper walls, heat being furnished by eight 25 watt nichrome heating elements, connected in parallel and placed around the pocket (L). Each heating element is in turn connected to a small platinum-tipped brass spring (B) which makes contact with a platinum point set on the ring (A). In this way one or more of the heating elements can be used as needed.

Temperature control is maintained by means of the sprung copper disc (D). When heat is applied to the liquid dichloromethane in (L), vapor is formed which will exert a pressure on the copper disc (D), tending to force it downward. This disc in turn presses against the pin (P) which when forced downward will cause the contact between (A) and (B) to be broken. In this way any or all of the heating elements (H) are switched in or off as needed to maintain a constant vapor pressure and temperature in the double walled space surrounding the chamber. By shifting the weight (W), tension on the disc (D) may be increased or decreased and the temperature in the chamber varied (within a certain range) at will. By selecting liquids having a boiling point higher than that of dichloromethane the temperature range may also be varied. Heating of the chamber by means of the vapor of a volatile liquid assures a uniform distribution of heat.

On using the apparatus for the first time the needle valve (V) is opened to allow air to escape and closed again when dichloromethane vapor begins to escape. This assures the space being filled solely with dichloromethane vapor. To fill or drain the liquid in (L) a drain plug (E) is provided. To insure rigidity and tightness, the walls are thoroughly braced and reinforced and all joints are silver-soldered.

The apparatus was constructed by Mr. A. J. Kercher of Berkeley and embodies several of his own inventions.

CHANGES IN THE H^+ AND OH^- CONCENTRATION WHICH TAKE PLACE IN THE FORMATION OF CERTAIN PROTEIN COMPOUNDS.

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(Received for publication, February 26, 1916.)

It has been known for some time that certain proteins combine with other proteins to form compound proteins. Kossel states that the protamines in weakly alkaline solutions combine with proteins to form compounds which precipitate under proper conditions. Kutscher,² working with albumoses obtained from Witte's peptone prepared compounds not only with protamine but also with various other proteins. While studying the properties of the histones, Bang³ was able to confirm the observations of Kutscher and further showed that various members of this group combine with proteins to form compounds. Kossel and Kutscher⁴ surmised that histones (on account of the high content of basic substances) might be a combination of protamine with other proteins but later Kossel and Pringle⁵ showed that this view was incorrect since in the first step in the peptic digestion of histones, histopeptone is formed, whereas protamine-protein combinations are split into protamine and protein. Hunter prepared a number of compounds of protamine with various proteins, such combinations as clupein-casein, clupein-gelatin, and clupein-edeitin being obtained. He further determined the proportion of the two substances which unite to form the compound protein. Gay and Robertson,⁷ studying the antigenic properties of

¹ Kossel, A., *Deutsch. med. Woch.*, 1894, xx, 147.

² Kutscher, F., *Z. physiol. Chem.*, 1897, xxiii, 115.

³ Bang, I., *Z. physiol. Chem.*, 1899, xxvii, 463.

⁴ Kossel, A., and Kutscher, F., *Z. physiol. Chem.*, 1900-01, xxxi, 165.

⁵ Kossel, A., and Pringle, H., *Z. physiol. Chem.*, 1906, xlix, 301.

⁶ Hunter, A., *Z. physiol. Chem.*, 1907, liii, 526.

⁷ Gay, F. P., and Robertson, T. B., *J. Exp. Med.*, 1912, xvi, 479.

compound proteins, prepared the compound protamine (salmine) caseinate. Robertson,⁸ unaware of the work of Bang, surmised from the possible relation of the histones to the protamines that globin might also unite with other proteins to form compounds. He prepared globin caseinate and determined its refractive index in $0.1\ N$ KOH solution. Later Gay and Robertson⁹ studied its antigenic properties.

It will be noted that in the work recorded precipitation of the compound took place in neutral or alkaline solutions, although Kossel¹ states that a nuclein may be formed in acid solution from the combination of nucleic acid and protein. Altmann¹⁰ had previously observed that proteins could combine in acid solution and Milroy¹¹ prepared a number of protein-nucleic acid compounds. Apparently no observations have been made to determine the true acidity (or alkalinity) of the protein solutions used or of the mixture when precipitation of the compound took place. The conditions for precipitation must be such that salting out of either of the proteins by inorganic salts formed during the reaction must not take place and the acidity of the solution must be such that the compound protein, if formed, will not be dissolved.

In the present work use of the gas chain has been made to follow the change of acidity (or alkalinity) of the solution during the precipitation of a compound protein to distinguish, if possible, salting out of either protein from true compound precipitation. Determination of the exact hydrogen ion concentration serves as a criterion for the duplication of any work in the preparation of compound proteins, since it appears that the exact acidity (or alkalinity) is a very important factor in the preparation of such compounds.

Use of the hydrogen electrode has been made by a large number of observers. Böttger¹² made use of the gas chain to determine the neutral point in the titration of acids and bases. The use of the hydrogen elec-

⁸ Robertson, T. B., *J. Biol. Chem.*, 1912-13, xiii, 499.

⁹ Gay and Robertson, *J. Exp. Med.*, 1913, xvii, 535.

¹⁰ Altmann, R., *Arch. Anat. u. Physiol., Physiol. Abt.*, 1889, 524.

¹¹ Milroy, T. H., *Z. physiol. Chem.*, 1896-97, xxii, 307.

¹² Böttger, W., *Z. physik. Chem.*, 1897, xxiv, 253.

trode for such purposes has been emphasized by Hildebrand,¹³ who points out its many applications in the analytical field. Salm,¹⁴ determining the H^+ concentrations in various phosphate mixtures, was able to prepare a scale of indicators for the colorimetric estimation of H^+ and OH^- concentrations. Schmidt and Finger¹⁵ showed that solutions of definite H^+ and OH^- concentrations may be made by using mixtures of various borates. They also point out that from the shape of the titration curve the existence of a compound in solution may be determined. Thus they confirmed observations made by various other methods by Noyes and Whitney,¹⁶ Kahlenberg and Schreiner,¹⁷ and Shelton¹⁸ that a compound NaH_2BO_3 , or the anhydride $NaBO_2$, or the corresponding ions H_2BO_3 and BO_2 exist in solution. In the field of protein chemistry much use has also been made of the hydrogen electrode. Thus Robertson¹⁹ used it to determine the dissociation of serum globulin at varying hydrogen ion concentrations, and the dissociation of potassium caseinate²⁰ and ovomucoid²¹ at varying alkalinities. Robertson and Schmidt²² were able to follow the change of alkalinity which took place during the progress of tryptic digestions of certain proteins. The many uses of the hydrogen electrode are set forth in a monograph by Michaelis.²³

The plan of these experiments was to determine the H^+ or OH^- concentration in a solution of protein "a". Definite amounts of either a solution of protein "b" or an inorganic salt used to precipitate protein "a" from its solution, were then added to the solution of protein "a". The H^+ concentration was then determined in the solution of protein "a" after each addition of the solution of protein "b". A titration curve can thus be plotted using cc. of solution "b" as ordinates and the resultant H^+ or OH^- concentrations as abscissae. In this way a titration curve is obtained similar to those described by Bottger,¹² Schmidt and Finger,¹⁵ and Hildebrand.¹³

¹³ Hildebrand, J. H., *J. Am. Chem. Soc.*, 1913, xxxv, 847.

¹⁴ Salm, E., *Z. Electrochem.*, 1904, x, 341; *Z. physik. Chem.*, 1907, lvii, 471.

¹⁵ Schmidt, C. L. A., and Finger, C. P., *J. phys. Chem.*, 1908, xii, 406. See also Hildebrand, J. H., and Bowers, W. G., *J. Am. Chem. Soc.*, 1916, xxxviii, 785.

¹⁶ Noyes, A. A., and Whitney, W. R., *Z. physik. Chem.*, 1894, xv, 694.

¹⁷ Kahlenberg, L., and Schreiner, O., *Z. physik. Chem.*, 1896, xx, 547.

¹⁸ Shelton, H. S., *Z. physik. Chem.*, 1903, xliii, 494.

¹⁹ Robertson, J. *phys. Chem.*, 1907, xi, 437.

²⁰ Robertson, J. *phys. Chem.*, 1910, xiv, 528.

²¹ Robertson, J. *phys. Chem.*, 1910, xiv, 709.

²² Robertson, and Schmidt, *J. Biol. Chem.*, 1908, v, 31.

²³ Michaelis, L., *Die Wasserstoffionenkonzentration*, Berlin, 1914.

The method used to determine the H^+ concentrations was essentially the same as used by Robertson²⁴ and Robertson and Schmidt,²⁵ and described by Schmidt and Finger¹⁵ and Robertson.²⁴ Hydrogen was generated by the electrolysis of a 6 per cent by volume solution of concentrated H_2SO_4 and passed over heated platinized asbestos to rid it of any oxygen or oxygen compounds. The hydrogen then passed through the solution at two points, through a nozzle at the bottom of the cell and

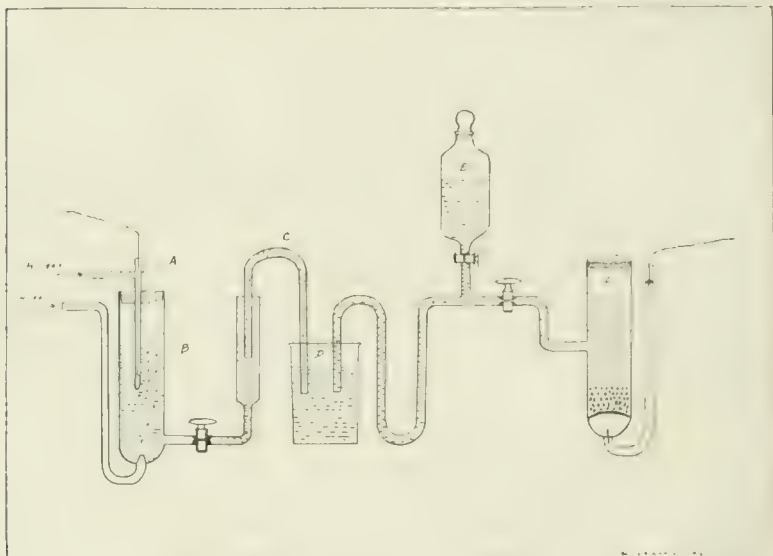


FIG. 1. A, platinum gauze electrode; B, titration cell; C, agar tube saturated with KCl; D, beaker with 0.1 N KCl; E, separatory funnel with 0.1 N KCl; F, 0.1 N KCl-HgCl-Hg-electrode.

through a Cottrell gauze electrode.^{15, 25} In this way the three phases, platinum, hydrogen, and solution were brought into intimate contact. A calomel electrode,²⁶ using 0.1 N KCl, was used as the other extremity of the chain. The apparatus is shown in Fig. 1. A is the gauze electrode dipping into the solu-

²⁴ Robertson, *Die physikalische Chemie der Proteine*, Dresden, 1912, 417.

²⁵ Robertson, *J. phys. Chem.*, 1907, xi, 442. Loomis, N. E., Dissertation, Johns Hopkins Univ., 1911, 22. Loomis, N. E., and Acree, S. F., *Am. Chem. J.*, 1911, xlv, 602.

²⁶ Richards, T. W., *Z. physik. Chem.*, 1897, xxiv, 39.

tion contained in cell B, D a beaker containing 0.1 \times KCl, and F the calomel electrode. Connection with cell B is made by means of an agar tube saturated with KCl which eliminates contact potential.^{27, 28} To eliminate contamination of the solution in cell B, the stop-cock connecting the side arm with the cell was kept closed except during the measurement of the potential and likewise the agar tube was dipped into the solution in the side arm only while readings were being taken. Through a hole in the stopper of cell B any amount of a solution "b" could be introduced. Measurement of the potential was made on a 100 cm. bridge using a sensitive Leeds and Northrup galvanometer²⁹ as zero instrument. Three Edison-Lalande cells connected in series furnished current through the potentiometer wire and the E.M.F. was checked against a Weston cell after each reading. The protein solutions "a" and "b" as well as the gauze electrode were separately saturated with hydrogen before bringing them together, the necessity for this having been shown by Robertson³⁰ and by Desha and Acree.³¹ Hydrogen was allowed to bubble through the solution from 45 to 60 minutes before determining the E.M.F., and this was likewise done after each addition of solution "b". All determinations were made at room temperature. To prevent foaming a few drops of octyl alcohol were floated on the surface of the solution in B after the introduction of the electrode. The H^+ and OH^- concentrations corresponding to the E.M.F.'s were taken from tables previously calculated by Schmidt.³¹

Since it is not feasible to determine H^+ or OH^- concentrations in ammoniacal solutions by means of the gas chain, the experi-

²⁷ Bjerrum, N., *Z. physik. Chem.*, 1905, liii, 428. Loomis, N. E., Dissertation, Johns Hopkins Univ., 1911, 34. Loomis, N. E., and Acree, S. F., *Am. Chem. J.*, 1911, xlv, 585. Clark, F. W., Myers, C. N., and Acree, S. F., *J. phys. Chem.*, 1916, xx, 241. Bjerrum, *Z. Electrochem.*, 1911, xvii, 389. See also Cumming, A. C., and Abegg, R., *Z. Electrochem.*, 1907, xiii, 17.

²⁸ This may not be strictly true, but since we are not concerned with absolute values but merely with changes in voltage this factor may be neglected.

²⁹ Kindly loaned by the Physics Department.

³⁰ Desha, L. J., and Acree, S. F., *Am. Chem. J.*, 1911, xlv, 638.

³¹ Schmidt, C. L. A., *Univ. California Publications, Physiology*, 1905-10, iii, 101.

ments were made with compounds of globin. As stated by Baig² and Robertson,³ precipitation of the compound protein takes place in slight excess of NaOH or KOH. Globin was prepared according to a modification of the method described by Robertson.³² Two preparations were made, one precipitated by NH_4Cl from an ammoniacal solution and the other by alcohol and ether from HCl solution. These correspond to Preparations I and II described by Robertson. Preparation II is soluble in water without addition of acid or alkali. The casein employed was Eimer and Amend's ("*nach Hammarsten*") which had been further purified according to Robertson.³³ Deuteroalbumose was made from Witte's peptone as described by Kutscher.² The nucleic acid was a preparation extracted by Dr. A. E. Taylor from the sperm of the Pacific Coast salmon (probably according to the method of Altmann).¹⁰ The bile salts were prepared from ox bile according to the method of Plattner.³⁴

The following is a tabular representation of the results obtained in the experiment. In the first column are given the volumes of the titrating solution "*b*" which were added to a given amount of solution "*a*". The second column shows the E.M.F. determined after the establishment of equilibrium. The H^+ and OH^- concentrations corresponding to the E.M.F.'s are given in the next two columns. In the last column are given the calculated possible errors in determining the H^+ concentration on the assumption that the determination of the E.M.F.'s was accurate to a millivolt.

Curves showing either the change in the H^+ or the OH^- concentration which took place during the titration as determined by experiment are plotted and given below. These will be referred to as titration curves. The first experiments were carried out to determine what effect was produced on the H^+ concentration by simple dilution and by salting out with those salts which might be formed as intermediate products of the reaction; so that, if possible, a distinction between salting out and true protein com-

³² Robertson, *J. Biol. Chem.*, 1912-13, xiii, 455.

³³ Robertson, *J. Biol. Chem.*, 1906-07, ii, 317; *J. phys. Chem.*, 1910, xiv, 534.

³⁴ Plattner, E. A., *J. prakt. Chem.*, 1847, xl, 129.

pound formation might be made. The results are given in Tables I, II, and III and shown graphically in Fig. 2. The change in H^+ concentration is a direct function of the titrating solution and the curves are straight lines, despite the fact that with both NH_4Cl and KCl a precipitate is obtained. In these instances we apparently have either a purely physical phenomenon or a chemical one which involves no sudden change in the H^+ concentration such as to give a break in the curve. The precipitation of proteins by inorganic salts has been studied extensively

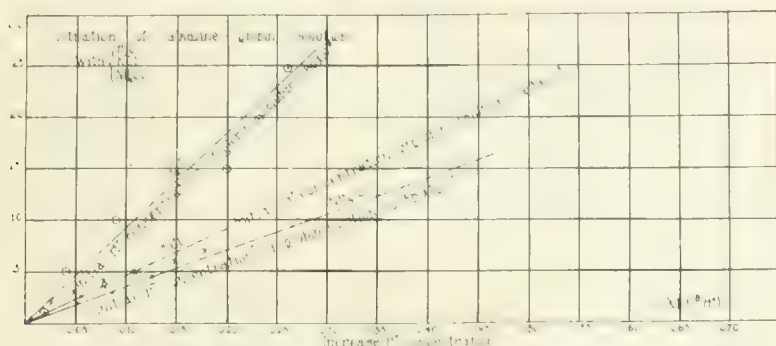


FIG. 2.

TABLE I.

Titration of Alkaline Globin. Preparation I. Solution with Water.

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. $0.1 \times KOH$, then titrated back with 7 cc. $0.1 \times HCl$. 50 cc. globin solution titrated. No precipitate produced.

H ₂ O added.	E.M.F.	H ⁺	OH ⁻	Possible error in H ⁺
cc.	volts			
0	0.861	0.80×10^{-9}	0.80×10^{-5}	$\pm 0.06 \times 10^{-9}$
1	0.856	0.98×10^{-9}	0.65×10^{-5}	0.08×10^{-9}
5	0.850	0.12×10^{-8}	5.3×10^{-6}	0.01×10^{-8}
10	0.842	0.17×10^{-8}	3.8×10^{-6}	0.01×10^{-8}
15	0.830	0.28×10^{-8}	2.3×10^{-6}	0.03×10^{-8}
20	0.827	0.31×10^{-8}	2.1×10^{-6}	0.02×10^{-8}
25	0.825	0.34×10^{-8}	1.9×10^{-6}	0.03×10^{-8}

70 Changes in H^+ and OH^- Concentration

TABLE II.

Titration of Alkaline Globin (Preparation I) Solution with a Saturated Solution of KCl.

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. 0.1 N KOH, then titrated back with 7 cc. 0.1 N HCl. 50 cc. globin solution titrated. Precipitate produced.

KCl solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.867	0.63×10^{-9}	1.0×10^{-5}	$\pm 0.05 \times 10^{-9}$
1	0.859	0.87×10^{-9}	0.74×10^{-5}	0.07×10^{-9}
2	0.859	0.87×10^{-9}	0.74×10^{-5}	0.07×10^{-9}
3	0.853	0.11×10^{-8}	5.8×10^{-6}	0.01×10^{-8}
4	0.847	0.14×10^{-8}	4.6×10^{-6}	0.01×10^{-8}
5	0.842	0.17×10^{-8}	3.8×10^{-6}	0.01×10^{-8}
6	0.837	0.21×10^{-8}	3.1×10^{-6}	0.02×10^{-8}
7	0.834	0.24×10^{-8}	2.7×10^{-6}	0.02×10^{-8}

TABLE III.

Titration of Alkaline Globin (Preparation I) Solution with a 0.25 Per Cent Solution of NH_4Cl .

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. 0.1 N KOH, then titrated back with 7 cc. 0.1 N HCl. 50 cc. globin solution titrated. Precipitate produced.

NH_4Cl solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.927	0.58×10^{-10}	1.1×10^{-1}	$\pm 0.05 \times 10^{-10}$
0.5	0.911	0.11×10^{-9}	5.8×10^{-5}	0.01×10^{-9}
1.0	0.893	0.22×10^{-9}	2.9×10^{-5}	0.01×10^{-9}
1.5	0.874	0.48×10^{-9}	1.3×10^{-5}	0.04×10^{-9}
2.0	0.869	0.58×10^{-9}	1.1×10^{-5}	0.04×10^{-9}
2.5	0.868	0.61×10^{-9}	1.1×10^{-5}	0.05×10^{-9}
3.0	0.857	0.94×10^{-9}	0.68×10^{-5}	0.07×10^{-9}
3.5	0.859	0.87×10^{-9}	0.74×10^{-5}	0.07×10^{-9}
4.5	0.848	0.13×10^{-8}	4.9×10^{-6}	0.01×10^{-8}
5.5	0.843	0.17×10^{-8}	3.9×10^{-6}	0.02×10^{-8}
7.5	0.835	0.23×10^{-8}	2.8×10^{-6}	0.02×10^{-8}
9.5	0.831	0.27×10^{-8}	2.4×10^{-6}	0.03×10^{-8}
14.5	0.819	0.43×10^{-8}	1.5×10^{-6}	0.03×10^{-8}

by Hardy,³⁵ Pauli,³⁶ Hofmeister,³⁷ Galeotti,³⁸ and many others, and is discussed at length by Robertson.³⁹

The titration of globin in acid and alkali solutions by alkali and acid respectively was next studied. For this purpose Preparation I, dissolved by a small amount of alkali, and Preparation II, which is water-soluble and acid in reaction, were used. During

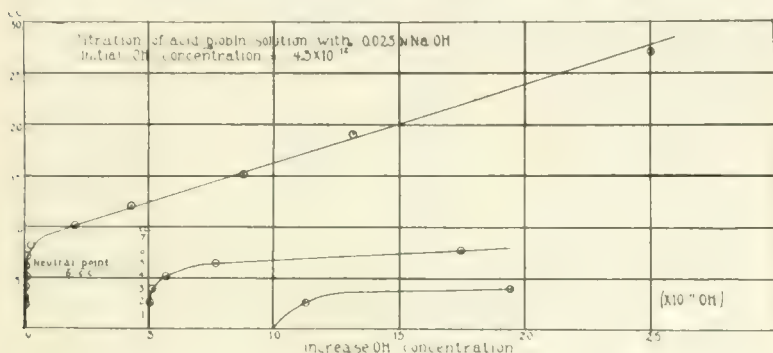


FIG. 3.

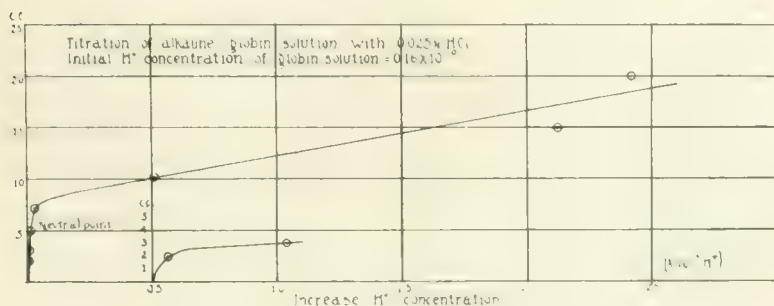


FIG. 4.

the titration the protein was precipitated and redissolved by excess of acid or alkali respectively. The results are given in

³⁵ Hardy, W. B., *J. Physiol.*, 1905-06, xxxiii, 251.

³⁶ Pauli, W., *Beitr. chem. Phys. u. Path.*, 1906, vii, 531.

³⁷ Hofmeister, F., *Arch. exp. Path.*, 1888, xxiv, 247; 1889, xxv, 1; 1890, xxvii, 395; 1891, xxviii, 210.

³⁸ Galeotti, G., *Z. physiol. Chem.*, 1903-04, xl, 492; 1904, xlii, 330.

³⁹ Robertson, *Die physikalische Chemie der Proteine*, Dresden, 1912, 84.

72 Changes in H^+ and OH^- Concentration

Tables IV and V and the changes in H^+ and OH^- concentrations graphically represented in Figs. 3 and 4. Since these reactions involve direct changes in H^+ and OH^- concentrations and

TABLE IV.

Titration of Acid Globin (Preparation II) Solution with 0.025 N NaOH.

Concentration of globin, 0.2 per cent. Globin dissolved in water without addition of acid or alkali. 50 cc. globin solution titrated. Precipitate produced which dissolved in excess of alkali.

NaOH solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	Vs.			
0	0.499	0.15×10^{-2}	4.3×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	0.534	0.37×10^{-3}	1.7×10^{-11}	0.02×10^{-3}
3	0.578	0.64×10^{-4}	1.0×10^{-10}	0.04×10^{-4}
4	0.632	0.75×10^{-5}	0.86×10^{-9}	0.03×10^{-5}
5	0.662	0.23×10^{-6}	2.8×10^{-8}	0.01×10^{-6}
6	0.757	0.51×10^{-7}	1.3×10^{-7}	0.02×10^{-7}
7	0.840	0.19×10^{-8}	3.5×10^{-6}	0.01×10^{-8}
8	0.893	0.22×10^{-9}	2.9×10^{-5}	0.01×10^{-9}
10	0.941	0.33×10^{-10}	2.0×10^{-4}	0.01×10^{-10}
12	0.961	0.15×10^{-10}	4.3×10^{-4}	0.01×10^{-10}
15	0.979	0.72×10^{-11}	0.89×10^{-3}	0.03×10^{-11}
19	0.989	0.48×10^{-11}	1.3×10^{-3}	0.02×10^{-11}
27	1.005	0.26×10^{-11}	2.5×10^{-3}	0.01×10^{-11}

TABLE V.

Titration of Alkaline Globin (Preparation I) Solution with 0.025 N HCl.

Concentration of globin, 0.2 per cent. Globin dissolved in 2 cc. 0.1 N NaOH, titrated back with 1.5 cc. 0.1 N HCl. 50 cc. globin solution used for titration. Precipitate produced which dissolved in excess of acid.

HCl solution added	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	Vs.			
0	0.959	0.16×10^{-10}	4.0×10^{-4}	$\pm 0.01 \times 10^{-10}$
2	0.920	0.76×10^{-10}	0.84×10^{-4}	0.03×10^{-10}
3	0.870	0.56×10^{-9}	1.2×10^{-5}	0.02×10^{-9}
5	0.747	0.76×10^{-7}	0.85×10^{-7}	0.03×10^{-7}
7	0.609	0.19×10^{-4}	3.4×10^{-10}	0.02×10^{-4}
10	0.525	0.53×10^{-3}	1.2×10^{-11}	0.02×10^{-3}
15	0.490	0.22×10^{-2}	3.0×10^{-12}	0.01×10^{-2}
20	0.487	0.24×10^{-2}	2.6×10^{-12}	0.01×10^{-2}

true compounds between protein and acid and alkali are formed, we obtain curves differing from those in which salting out of protein has taken place. The titration curves of acid and alkaline globin are very similar to the titration curve of hydrochloric acid by sodium hydroxide given by Böttger⁴⁰ and cited by Hildebrand,⁴¹ except that a different neutral point is obtained. This is to be expected, since globin can act either as an acid or a base. Thus in titrating acid globin with alkali, on approaching the neutral point, globin will become acid and will neutralize alkali. The sudden shift in the slope of the curve near the neutral point indicates compound formation and serves as a criterion for the determination of compound formation, whether the compound is precipitated or remains in solution. This will be shown even better in other curves. That the first part of the curve is not a straight line but appears to be so, since the changes in H^+ or OH^- concentration are small as compared with the rest of the curve, is shown on magnifying the first portion of the titration curve.

TABLE VI.

Titration of Alkaline Globin (Preparation I) Solution with a Solution of Casein (Neutral to Phenolphthalein).

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. 0.1 N KOH, then titrated back with 6.9 cc. 0.1 N HCl. Concentration of casein in titrating solution, 0.5 per cent. 0.25 gm. casein dissolved by 4 cc. 0.1 N KOH, then titrated back with 2 cc. 0.1 N HCl. 50 cc. globin solution titrated. No precipitate produced.

Casein solution added	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.894	0.21×10^{-9}	3.0×10^{-5}	$\pm 0.02 \times 10^{-9}$
0.5	0.892	0.23×10^{-9}	2.8×10^{-5}	0.02×10^{-9}
1.5	0.888	0.27×10^{-9}	2.4×10^{-5}	0.02×10^{-9}
2.5	0.883	0.33×10^{-9}	1.9×10^{-5}	0.02×10^{-9}
3.5	0.880	0.38×10^{-9}	1.7×10^{-5}	0.03×10^{-9}
8.5	0.869	0.58×10^{-9}	1.1×10^{-5}	0.04×10^{-9}
13.5	0.861	0.80×10^{-9}	0.80×10^{-5}	0.06×10^{-9}
18.5	0.848	0.13×10^{-8}	4.8×10^{-6}	0.01×10^{-8}
23.5	0.836	0.22×10^{-8}	3.0×10^{-6}	0.02×10^{-8}
28.5	0.825	0.34×10^{-8}	1.9×10^{-6}	0.03×10^{-8}

⁴⁰ Böttger, W., *Z. physik. Chem.*, 1897, xxiv, 281.

⁴¹ Hildebrand, J. H., *J. Am. Chem. Soc.*, 1913, xxxv, 854.

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TABLE VII.

Titration of Alkaline Globin (Preparation I) Solution with a Solution of Casein Neutral to Litmus.

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. 0.1 N KOH, then titrated back with 7.1 cc. 0.1 N HCl. 50 cc. globin solution titrated. Concentration of casein in titrating solution, 0.5 per cent. 0.25 gm. casein dissolved by 3 cc. 0.1 N KOH, then titrated back with 1.5 cc. 0.1 N HCl. Slight precipitate produced.

Casein solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.843	0.17×10^{-8}	3.9×10^{-6}	$\pm 0.02 \times 10^{-8}$
1	0.833	0.24×10^{-8}	2.6×10^{-6}	0.01×10^{-8}
2	0.831	0.26×10^{-8}	2.4×10^{-6}	0.02×10^{-8}
3	0.821	0.40×10^{-8}	1.6×10^{-6}	0.03×10^{-8}
4	0.820	0.41×10^{-8}	1.6×10^{-6}	0.03×10^{-8}
5	0.818	0.45×10^{-8}	1.4×10^{-6}	0.04×10^{-8}
10	0.804	0.78×10^{-8}	0.82×10^{-6}	0.06×10^{-8}
15	0.803	0.81×10^{-8}	0.79×10^{-6}	0.06×10^{-8}
25	0.801	0.88×10^{-8}	0.73×10^{-6}	0.07×10^{-8}

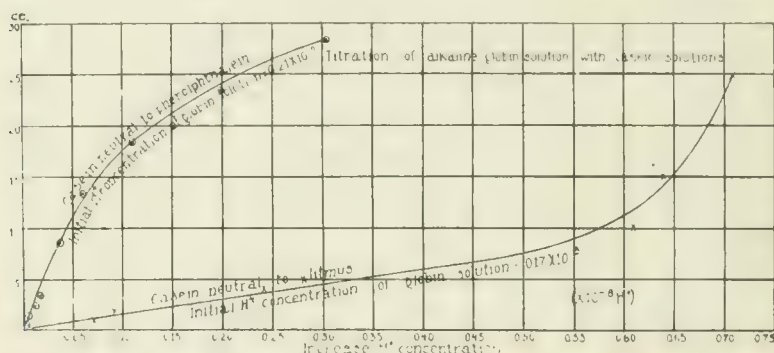


FIG. 5.

As previously stated, both Bang and Robertson obtained compounds of globin and casein which they state were precipitated in alkaline solution. On attempting to duplicate this work, no precipitate was obtained though a compound between globin and casein is formed as shown by the data in Tables VI and VII and represented graphically in Fig. 5. The compound formed is apparently soluble. However, on titrating an acid solution of

globin with a solution of casein (neutral to phenolphthalein) a precipitate was obtained and the titrating curve (see data in Table VIII and Fig. 6) shows that a compound was formed.

TABLE VIII.

Titration of Acid Globin (Preparation II) Solution with a Solution of Casein.

Concentration of globin, 0.2 per cent. Globin dissolved in water and 1 cc. 0.1 \times NaOH added. Concentration of casein (neutral to phenolphthalein), 0.5 per cent. 50 cc. globin solution titrated. Precipitate produced.

Casein solution added.	E.M.F.	H ⁺	OH ⁻	Possible error in H ⁺
cc.	volts			
0	0.552	0.18×10^{-3}	3.5×10^{-11}	$\pm 0.01 \times 10^{-3}$
1	0.577	0.67×10^{-4}	0.96×10^{-11}	0.03×10^{-4}
2	0.609	0.19×10^{-4}	3.4×10^{-10}	0.01×10^{-4}
4	0.614	0.15×10^{-4}	4.2×10^{-10}	0.01×10^{-4}
6	0.623	0.11×10^{-4}	6.0×10^{-10}	0.01×10^{-4}
8	0.637	0.61×10^{-5}	1.1×10^{-9}	0.03×10^{-5}
11	0.650	0.36×10^{-5}	1.8×10^{-9}	0.02×10^{-5}
16	0.685	0.90×10^{-6}	0.71×10^{-8}	0.04×10^{-6}
21	0.707	0.37×10^{-6}	1.7×10^{-8}	0.03×10^{-6}

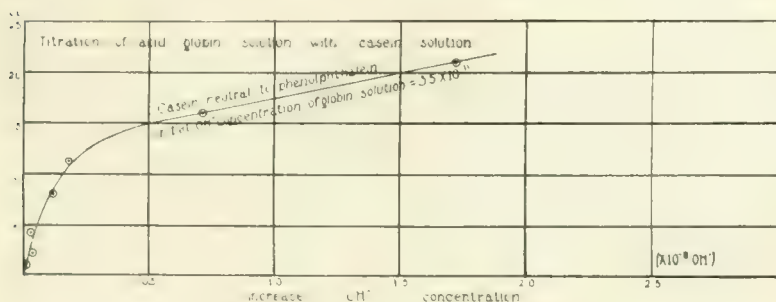


FIG. 6.

Apparently Robertson had used Globin Preparation II, which, being precipitated from an acid solution by alcohol and ether, was the acid compound of globin; and by dissolving in weak alkali, Robertson had merely partly neutralized the acid combined with the globin (not enough to cause precipitation of the globin) so that his solution was still acid, instead of alkaline as one is led to believe from his description. This was likewise true of the work

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of Bang, who prepared globin according to the method of Schulz.¹ Determination of the initial acidity of the globin solution would have served as a criterion for the duplication of the work.

TABLE IX.

Titration of Acid Globin Preparation II Solution with a Solution of Sodium Nucleate.

Concentration of globin, 0.2 per cent. Globin dissolved in water without addition of acid or alkali. Concentration of nucleic acid, 0.4 per cent. Nucleic acid neutralized by NaOH. 50 cc. globin solution titrated. Precipitate produced.

Sodium nucleate solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.488	0.23×10^{-2}	2.8×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	0.486	0.25×10^{-2}	2.5×10^{-12}	0.01×10^{-2}
4	0.504	0.12×10^{-2}	5.2×10^{-12}	0.01×10^{-2}
8	0.525	0.53×10^{-3}	1.2×10^{-11}	0.02×10^{-3}
12	0.536	0.34×10^{-3}	1.9×10^{-11}	0.02×10^{-3}
16	0.560	0.13×10^{-3}	4.9×10^{-11}	0.01×10^{-3}
20	0.572	0.82×10^{-4}	0.78×10^{-10}	0.03×10^{-4}
25	0.585	0.49×10^{-4}	1.3×10^{-10}	0.03×10^{-4}

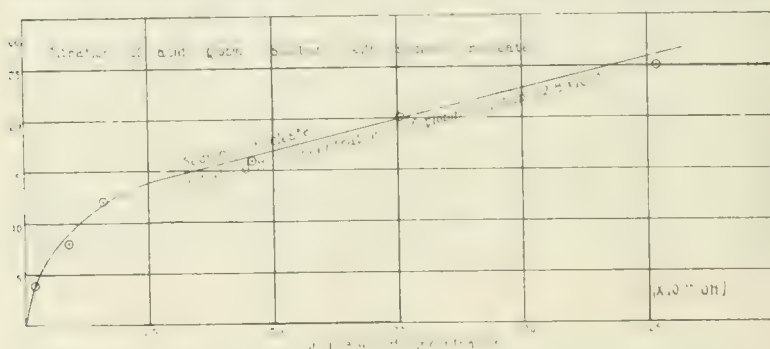


FIG. 7.

Globin also forms compounds with nucleic acid and taurocholic acid, precipitation taking place in an acid solution. The titration curve of globin with nucleic acid (see Table IX and Fig. 7) is very similar to that obtained on titration of acid glo-

¹ Schulz, F. N., *Z. physiol. Chem.*, 1898, xxiv, 449.

bin with casein. The titration curve of acid globin with bile salts (see Tables X and XI, and Fig. 8) shows a sharp break at the

TABLE X.

Titration of Acid Globin (Preparation II) Solution with a Solution of Bile Salts.

Concentration of globin, 0.2 per cent. Globin dissolved in water without addition of acid or alkali. Concentration of bile salts in aqueous solution, 0.4 per cent. Precipitate produced.

Bile salts solution added.	E.M.F.	H ⁺	OH ⁻	Possible error in H ⁺
cc.	volts			
0	0.484	0.27×10^{-2}	2.3×10^{-12}	$\pm 0.01 \times 10^{-2}$
2	0.498	0.16×10^{-2}	4.1×10^{-12}	0.01×10^{-2}
4	0.503	0.13×10^{-2}	5.0×10^{-12}	0.01×10^{-2}
5	0.507	0.11×10^{-2}	5.9×10^{-12}	0.01×10^{-2}
7	0.511	0.93×10^{-3}	0.69×10^{-11}	0.04×10^{-3}
10	0.508	0.11×10^{-2}	6.1×10^{-12}	0.01×10^{-2}
13	0.512	0.89×10^{-3}	0.72×10^{-11}	0.04×10^{-3}
16	0.517	0.73×10^{-3}	0.88×10^{-11}	0.03×10^{-3}
24	0.540	0.29×10^{-3}	2.2×10^{-11}	0.01×10^{-3}

TABLE XI.

Titration of Acid Globin (Preparation II) Solution with a Solution of Bile Salts.

Concentration of globin, 0.2 per cent. Globin dissolved in water without addition of acid or alkali. Concentration of bile salts in aqueous solution, 0.4 per cent. Several drops 0.1 N NaOH added to the solution of bile salts to make it slightly alkaline. Precipitate produced during titration.

Bile salts solution added.	E.M.F.	H ⁺	OH ⁻	Possible error in H ⁺
cc.	volts			
0	0.515	0.79×10^{-3}	0.81×10^{-11}	$\pm 0.04 \times 10^{-3}$
2	0.521	0.63×10^{-3}	1.0×10^{-11}	0.03×10^{-3}
5	0.525	0.53×10^{-3}	1.2×10^{-11}	0.02×10^{-3}
8	0.524	0.55×10^{-3}	1.2×10^{-11}	0.02×10^{-3}
10	0.526	0.51×10^{-3}	1.3×10^{-11}	0.02×10^{-3}
13	0.532	0.40×10^{-3}	1.6×10^{-11}	0.02×10^{-3}
17	0.536	0.34×10^{-3}	1.9×10^{-11}	0.02×10^{-3}
21	0.542	0.27×10^{-3}	2.4×10^{-11}	0.01×10^{-3}
25	0.548	0.21×10^{-3}	3.0×10^{-11}	0.01×10^{-3}

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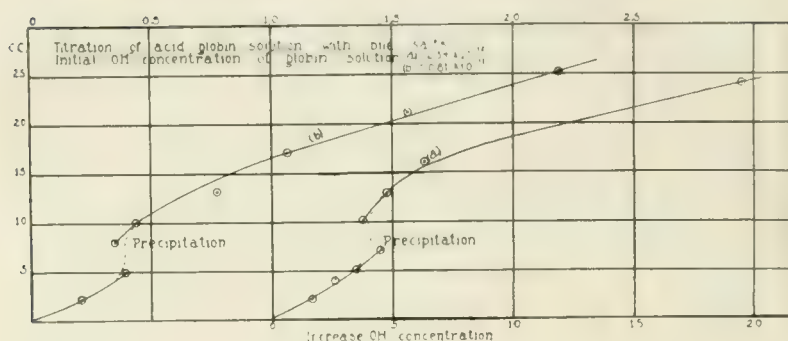


FIG. 8.

point where the compound protein starts to precipitate, and the slope of the curve changes. This is to be expected since we are titrating with a mixture of two salts, sodium taurocholate and sodium glycocholate. Globin also forms a compound with deutoalbumose, the latter acting as a weak acid. The titration curve (Fig. 9 and Table XII) indicates the formation of a compound, the proportion being roughly two parts of globin to one of albumose.

TABLE XII.

Titration of Alkaline Globin (Preparation I) Solution with a Solution of Deutoalbumose.

Concentration of globin, 0.5 per cent. 0.25 gm. globin dissolved by 8 cc. 0.1 N KOH, then titrated back with 7.1 cc. 0.1 N HCl. Concentration of deutoalbumose, 0.6 per cent. 50 cc. globin solution titrated. Precipitate produced.

Albumose solution added.	E.M.F.	H^+	OH^-	Possible error in H^+
cc.	volts			
0	0.835	0.23×10^{-8}	2.8×10^{-6}	$\pm 0.02 \times 10^{-8}$
1	0.813	0.54×10^{-8}	1.2×10^{-6}	0.04×10^{-8}
2	0.792	0.13×10^{-7}	5.1×10^{-7}	0.01×10^{-7}
4	0.793	0.12×10^{-7}	5.3×10^{-7}	0.01×10^{-7}
7	0.792	0.13×10^{-7}	5.1×10^{-7}	0.01×10^{-7}
15	0.786	0.16×10^{-7}	4.0×10^{-7}	0.01×10^{-7}
20	0.779	0.21×10^{-7}	3.0×10^{-7}	0.01×10^{-7}

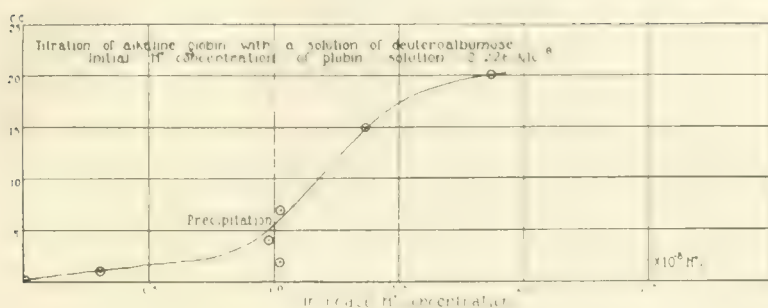


FIG. 9.

SUMMARY.

1. Use of the gas chain has been made to follow the changes in the H^+ and OH^- concentration in titrating acid and alkaline solutions of globin with solutions of other proteins and of precipitating inorganic salts.

2. Titration curves showing the changes in H^+ and OH^- concentration during titration have been plotted which indicate that true protein compounds of globin are formed, some of which may precipitate at a proper acidity or alkalinity, while others may be soluble.

3. A distinction can be made by means of the gas chain between the salting out of globin from solution by inorganic salts and the precipitation of a compound protein.

4. Determination of the H^+ or OH^- concentration in protein solutions used in the preparation of compound proteins serves as a criterion for the duplication of such work.

I am indebted to Professors F. P. Gay and T. Brailsford Robertson for the interest taken in this work, and to the George Williams Hooper Foundation for Medical Research for financial aid in carrying out the investigation.

INTESTINAL OBSTRUCTION

A PROTEOSE INTOXICATION

G. H. WHIPPLE, M.D.

SAN FRANCISCO

INTESTINAL OBSTRUCTION

A PROTEOSE INTOXICATION *

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The poison under discussion may be obtained from the fluid above an intestinal obstruction, from a closed washed loop of small intestine or from the mucosa of a closed loop or a loop draining externally through an enterostomy wound. The intoxication which develops with intestinal obstruction or after producing a closed or drained intestinal loop is very similar, differing only in intensity. In like manner one can inject this poison into a dog and produce more or less profound intoxication, depending on the amount of poison introduced. This intoxication resembles closely that observed following intestinal obstruction. The evidence is overwhelming that an active poison is concerned in the production of the clinical picture of intestinal obstruction and of the closed intestinal loops.

The poison is found in rather concentrated form within the lumina of the closed loops of the small intestine. Dogs can be immunized by sublethal doses and can then withstand lethal doses of this poison, but the immunity cannot be carried very high. Moreover, these immune dogs can survive intestinal obstruction or closed loops much longer than controls. This immunity is associated with a developing capacity of the organ cells of the body to destroy this toxic substance. Filtered immune organ extracts, presumably containing active enzymes, can destroy this poison in vitro, but the blood of immune animals

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* From the George Williams Hooper Foundation for Medical Research. This work was begun in Baltimore with the cooperation of Drs. Stone and Bernheim and continued in San Francisco with the assistance of Drs. Cooke and Rodenbaugh. The detailed experiments will be published in the near future.

contains no such enzymes and is inactive toward the poison.

If a loop of small intestine is washed out thoroughly, its ends closed and the continuity of the intestine established around it by means of a gastro-enterostomy or entero-enterostomy, any intoxication which may ensue must be due to one of two things: (1) bacterial activity; (2) perverted activity of the loop mucosa (or both factors in conjunction).

We have been able to show conclusively that the intestinal mucosa is essential to the production of this poison because with destruction of the mucosa by means of sodium fluorid the formation of the poison ceases. Whether bacterial activity is essential to the production of this poison is less certain, but no conclusive evidence that bacteria are essential has been brought forward. It is certain that bacteria alone cannot form this poison.

Absorption of this poison from the intestine raises a very interesting and perplexing point. When it is noted that the poisonous substance is most abundant and easily demonstrated in the lumina of closed loops, one's first thought is that absorption must be mainly from the lumen, but this is not so. Some absorption may take place from the contents of the loop, particularly if the mucosa is damaged or inflamed, but fatal intoxication will usually result from a loop draining externally and washed daily. Moreover, the introduction of large amounts of poison into a loop will not alter the course of the intoxication and such animals show no more intoxication than controls with loops washed and left empty at the operation. The poison is not absorbed from the normal intestine. All evidence indicates that the absorption of the poison in intestinal obstruction or in closed loops will take place mainly from the mucosa regardless of the material in the lumen of the gut. Our attention should center on the mucous membrane which forms this poison and passes it into the blood stream. This pernicious activity may be carried on to a fatal termination by a mucosa which may be to the naked eye as well as the microscope quite normal except for capillary engorgement.

The *chemical nature* of this poison is of great interest, because once known it will be possible to con-

concentrate attention on the action of similar poisons and finally uncover the best method of the body defense and cure. It is a very elusive substance and necessarily concealed in a mass of confusing materials. Many workers have hazarded guesses about this poison but it has never been isolated in anything like purity or identified. Almost all of the conflicting statements about this poison can be easily explained when it is realized that it is a primary proteose. We have been able to show that it is thrown out of a solution rich in albumins by boiling (adsorption) but that after autolysis or digestion it may be boiled without harm. Its proteose nature explains why the poison is held back in fine earthen filters, as stated by other observers, but not held back by paper filtration. The poison is not destroyed by digestion with pancreatin or fresh pancreas for a period of one week. It is not destroyed by autolysis with intestinal mucosa for a space of weeks but may be completely destroyed in months. Its reaction after intravenous injection in dogs closely simulates anaphylactic shock in these animals, but the poison cannot sensitize guinea-pigs for the characteristic anaphylactic phenomena.

Alcohol precipitates this poison and this is a most important step in the separation of this substance from the mass of extraneous material in the intestinal loop fluid. Five volumes of 95 per cent. alcohol will precipitate completely all of this poison, whether the fluid is freshly removed from an intestinal loop rich in albumins or has been digested to a clear broth. The alcoholic filtrate has been tested repeatedly and contains not a trace of the poison, but does contain the substance which produces the short initial fall in blood pressure (the "tissue extract" reaction). The alcoholic precipitate contains all of the poison. This precipitate may be extracted with ether for days without removing any of the poison and the ether extract gives no reaction when given intravenously in dogs. Water will remove all of the poison from the alcoholic precipitate and the poison goes into solution very rapidly. The residue may be filtered off, giving a clear opalescent solution. This of course contains some albumin, which may be removed in great measure by boiling in faintly acid reaction, and filtration. The

resultant filtrate contains the poison, as shown by repeated experiments.

The poison may be still further purified by precipitation at room temperature with equal parts of a saturated solution of ammonium sulphate. This gives a delicate precipitate which may be collected by centrifugalization, dried between filter paper, again dissolved in water and boiled with a drop of acetic acid to remove the last trace of albumin. This gives a solution almost water clear but slightly opalescent. Dialysis of this pure solution against normal salt will remove the most of the ammonium sulphate but will not change the toxic action of the poison, which does not dialyze in the least. This pure solution gives biuret precipitates with phosphotungstic acid or saturation with sodium chlorid. Estimations of dry weight show that 100 mg. of this pure poison is sufficient, if given intravenously, to poison fatally a 15-pound dog.

This process of chemical isolation gives a *primary proteose* and eliminates practically all other substances except sodium chlorid and a trace of ammonium sulphate. Because of the fact that this proteose resists digestion it may be classed as a heteroproteose. The symptoms of proteose intoxication are identical with those of poisoning by loop fluid, and sublethal doses of other proteoses will immunize against this poison and prolong the life of dogs with closed intestinal loops, beyond the controls. The anatomic picture is the same in fatal proteose intoxication and in poisoning with loop fluid. We may assume with certainty that the intoxication of intestinal obstruction and closed intestinal loops is due in great measure to the absorption of a primary proteose from the intestinal mucosa.

It has been shown by Chittenden and Mendel that proteose injected into the blood may be excreted with some rapidity in the urine. We have not been able to detect this substance in the urine perhaps because of the small amount which is fatally toxic and because the flow of urine is inhibited after a large dose. The proteose may be changed in some manner before it is eliminated. Dogs with a trace of albumin in the urine usually show some proteose by Hammarsten's method and this introduces a confusing factor.

It is obvious that diuresis will help in the elimination of this toxic proteose and this explains the beneficial effect of salt infusions in intestinal obstruction. This brings up another point of some interest and of considerable importance from the standpoint of diagnosis.

The injection of this toxic proteose causes a great rise in the incoagulable nitrogen of the blood, which shows a curve rising with the intensity of the intoxication from a normal 30 mg. to 70 or 80 mg. shortly before death, and this change may take place in acute poisoning within a space of three hours.

Dogs with intestinal obstruction may show an incoagulable nitrogen as high as 200 mg. and about the same is true of the closed-loop dogs. The rise in incoagulable nitrogen of the blood seems to depend on the intensity and rapidity of the intoxication and is of considerable prognostic and diagnostic value. Tilleston has reported two observations on human cases with very high incoagulable nitrogen of the blood in intestinal obstruction.

In conclusion it is to be emphasized that the intoxication of intestinal obstruction and closed intestinal loops is due to the absorption from the intestinal mucosa of a *primary proteose*. This proteose intoxication may be associated with a rise in the incoagulable nitrogen of the blood to four or even ten times normal. This fact may be of value in diagnosis and prognosis.

University of California.

INTESTINAL OBSTRUCTION.

V. PROTEOSE INTOXICATION.

By G. H. WHIPPLE, M.D., F. H. RODENBAUGH, M.D., AND
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(Received for publication, July 21, 1915.)

In earlier publications Whipple, Stone, and Bernheim (1, 2, 3, 4) have established facts which have an important bearing on this question of proteose intoxication. Some of these points will be reviewed, but for the detailed experiments the original papers should be consulted. In this work closed loops of intestine have been used because it has been established by previous experiments that an acute intoxication develops, and the toxic substance may be obtained in concentrated form in the material which accumulates in the closed loops. The intoxication noted in simple intestinal obstruction is similar, but usually less intense than that observed in dogs with closed or drained duodenal loops. Much evidence has been furnished to demonstrate that the essential toxic substance is very similar or even identical in obstruction and in closed intestinal loops.

All recent work speaks strongly for the theory of a definite intoxication in intestinal obstruction and closed loops of intestine. The doubts expressed by Hartwell and Hogue (5) have been answered by Draper (6). Hartwell and Hogue claimed that loss of fluid and consequent dehydration of the tissues were primary and the intoxication a secondary result of this dehydration. Whipple, Stone, and Bernheim have claimed that the intoxication was primary and the dehydration of the tissues secondary to the intoxication. Draper has been able to show that a dog can be dehydrated in four days by pilocarpin to the same degree encountered in duodenal obstruction. One of us has been able to bring about a similar dehydration and blood concentration by means of pilocarpin and vigorous salt purgation

over a period of thirty-six hours. The dogs which are dehydrated by means of drugs show no evidence of intoxication and recover their weight very promptly when given abundant food and water. It is safe to assume that there is a primary intoxication in intestinal obstruction and with closed intestinal loops.

When a loop of small intestine is washed out, closed by tapes, and a gastro-enterostomy made to reestablish the continuity of the intestine, we may attribute any resultant intoxication to one or both of two factors: (1) bacteria, and (2) intestinal mucosa. We have shown elsewhere that destruction of the mucosa prevents the formation of any toxic substance in the loop; in other words, the mucosa is essential to the production of the poison. Whether bacteria are essential or not is more difficult of proof and this point has not been established as yet beyond criticism. Murphy and Brooks (7) submit an experiment which suggests that bacteria are essential, but it may be claimed that the mucosa of a sterile drained loop, probably in a dog more or less strongly immunized, might not react in the usual manner. The poison formed in this inoculated loop did not give the usual reaction when given intravenously in a normal dog.

It has been claimed by Whipple, Stone, and Bernheim that the poison can be formed in a closed loop with no demonstrable lesions in the mucosa. This has been disputed by Hartwell and Hoguet (5, 8), and by Murphy and Brooks (7). It may be admitted that commonly the fluid accumulates in the closed loops, that this causes pressure and interference with the blood supply and finally injury to the mucosa. Some cases, however, are found in which only a little pasty material is found in a closed loop immediately after death. We wish to emphasize the fact that in such cases immediately after death we are unable to find the slightest histological evidence of injury to the mucosa. It is not necessary to find histological evidences of abnormality to support our belief that this mucous membrane is abnormal in that it forms a toxic proteose and allows its escape into the blood stream. We are convinced that these cells can present an abnormal physiological activity without any demonstrable histological change.

Absorption of this poison from the intestinal tract brings up some points of much practical importance. It is probable that injury of the mucosa will greatly facilitate toxic absorption, but it must be

remembered that the normal mucosa can not absorb any of the poison. Further, it is known that closed loops may be filled with much toxic material without giving any increase in intoxication. Dogs whose loops are filled with a lethal dose of toxin will live as long as control dogs whose loops are left empty at the end of the operation. Drained loops which are washed frequently give ample evidence of intoxication. This shows that much, if not the greater part of the intoxication is due to absorption from the mucous membrane alone rather than from the material in the lumen of the gut. Our attention should be concentrated on the mucous membrane in an effort to discover the fundamental change which is responsible for the toxin production and the reaction which may be responsible for its sudden return to normal activity.

The chemical nature of this poison is of great interest and of some practical importance. When its identity is established it will be possible to study all similar intoxications with the possibility of discovering the best means of combating the intoxication or neutralizing the poison or helping in its elimination. The fundamental objective of course is to check the formation of the poison at its source, but this may be impossible.

It is a very elusive substance and of necessity concealed in a mass of confusing material. Most workers in this field have hazarded a guess as to the identity of the poison. Albeck (9) believed that it belonged in the group of putrefactive poisons. Murphy and Brooks (7) believe that it may be similar to the substance sepsin isolated by Faust (10). It has been suggested that it might resemble the β -iminazoly ethylamine of Dale (11). Some of the reactions of this closed loop poison resemble those produced by the anaphylatoxin.

As evidence for its chemical analysis and identification it is to be recalled that this substance is not capable of sensitizing guinea pigs for the characteristic anaphylactic reaction. Dogs can be immunized to the poison, but this protection is not great, and an immune animal can scarcely withstand a double lethal dose. The immunity does not reside in the body fluids which are inert toward the poison. The immune organs or the autolyzed tissue juices contain ferments which destroy the poison *in vitro* (Whipple, Stone, and Bernheim (3)).

It is to be recalled that this poison may be thrown out of a rich

albuminous solution by boiling. The poison is more or less completely removed by Berkefeld filters (Murphy and Vincent (12) and Kukula (13)). Our earlier experiments show that autolysis with normal intestinal mucosa for a week will not destroy the poison (2). Long autolysis with mucosa weakens its toxic power and after many months (8 to 12) the poison may be completely destroyed. This resistance to digestive enzymes is characteristic and of considerable importance.

EXPERIMENTAL OBSERVATIONS.

The experiments given below show that the poison is not destroyed by pancreatic digestion for seven days. The most important observation, that alcohol precipitates this poison, is of great help in the isolation of this substance. Five volumes of 95 per cent alcohol will cause complete precipitation of the poison, and the filtrate is free from the toxic substance. This point has been established beyond a doubt by repeated experiments, and in this way a great number of confusing substances may be eliminated. Further purification may be effected by solution of the alcoholic precipitate and reprecipitation by one-half saturation with ammonium sulphate. Dialysis may be used, as the toxin does not pass a collodion membrane. The pure substance is a primary proteose, and may be classed as a hetero-proteose because it resists digestion. It is very toxic, and 100 mg. may be sufficient to poison a fifteen pound dog with the characteristic fatal reaction.

Methods.

Dogs were used in all the experiments. The kymograph observations were taken from the carotid artery exposed by a small incision at the root of the neck. In the abdominal operations all the usual aseptic precautions were observed. Ether anesthesia was used in all experiments. Hammarsten's method for testing the urine for proteoses may be outlined as follows. Equal parts of urine and saturated ammonium sulphate are heated to a boil. The precipitate is thrown down in a centrifuge, the supernatant fluid decanted, and the precipitate washed repeatedly with alcohol to remove the urobilin. The residue is dissolved in water, boiled, and filtered to remove albumin. The solution is extracted with chloroform and finally tested with the biuret.

Poison Not Destroyed by Complete Pancreatic Digestion.

Duodenal loop fluid (30 cc., a known lethal dose) was put away in the incubator at 38° C. after mixing with a freshly prepared dog's pancreas. The pancreas had been ground with sand, allowed to stand for 1 to 2 hours mixed with the loop fluid, made alkaline to litmus, and diluted to a thin soup. The mixture was protected from putrefaction by chloroform and toluol. Digestion proceeded for 7 days. There was no unpleasant odor, and the mixture was almost wholly fluid. It was passed through a thick Gooch crucible filter giving a clear amber fluid which was used to inject into a normal dog (Dog S-16).

Dog S-16.—Small adult mongrel, male; weight 14½ pounds.

Feb. 17, 3 p.m. Intravenous injection of the clear filtrate just described (30 cc.). This gave the usual reaction seen after the injection of the toxic material into dogs. The initial fall in blood pressure was transient with rapid return to normal. The progressive lasting fall in pressure began at the end of 30 minutes. 3.30 p.m. Dog is shocked and shows air hunger. 8.00 p.m. Death with extremely low temperature.

Autopsy.—The findings are typical of death by a large dose of the poison found in closed duodenal loops. Splanchnic engorgement and fluid in the intestines are the striking features.

Poison Precipitated by Alcohol and Soluble in Water.

Duodenal loop fluid (40 cc.), precipitated by three volumes of 95 per cent alcohol and heated for an hour over a boiling water bath, was filtered.

1. The filtrate or alcoholic extract was evaporated slowly in a vacuum at 80° C. to a small volume and tested on a normal dog with negative results. Kymograph tracings taken during the injection showed a prompt initial fall in blood pressure with return to normal in one or two minutes. The substance which causes the initial fall in blood pressure similar to the reaction of tissue extracts is soluble in alcohol. The dog recovered without showing the least signs of intoxication.

2. The precipitate was extracted with ether for several days and the ether extract allowed to evaporate, giving a fatty residue. This ether residue was taken up in alcohol and tested on a normal dog with negative result.

3. The precipitate (extracted by alcohol and ether) was finally extracted with water over a water bath, and the watery filtrate obtained by rapid paper filtration. It was tested on Dog S-39. Fatal poisoning in three and one-half hours.

4. The residue was finally digested with intestinal mucosa effecting a complete solution. This fluid was tested on a normal dog with negative results.

Dog S-39.—Small yellow mongrel, male; weight 13½ pounds.

Apr. 19, 11 a.m. Intravenous injection of watery extract of the alcoholic precipitate from the duodenal loop fluid. This caused some initial drop in blood pressure, followed by a return to normal lasting during the kymograph observa-

tion. 1.30 p.m. Dog is gravely shocked; much diarrhea and vomiting. 2.30 p.m. Death in collapse. Rectal temperature 30° C.

Autopsy.—The findings are typical of acute poisoning with duodenal loop fluid. Splanchnic congestion very marked. Blood concentration is striking; dry weight 26.3 per cent.

Alcoholic Precipitate Contains Poison.

Duodenal loop fluid (30 cc.) was allowed to digest with a little intestinal mucosa plus chloroform for over two weeks. This gave a clear broth-like filtrate which contains the poison in such experiments. This clear solution was treated with five volumes of 95 per cent alcohol, and the mixture was allowed to stand for three days after being brought to a boil over a water bath. A very scanty precipitate came down on standing. This precipitate was collected on a small filter and washed with alcohol and ether. It was estimated, by comparison with fibrinogen precipitates collected in similar manner, at 30 to 60 mg. This precipitate was completely dissolved in water and used for intravenous injection as before.

Dog O-74.—Small mongrel, female; weight 8½ pounds.

Apr. 21, 2.30 p.m. Intravenous injection of the above preparation caused no initial drop in blood pressure, but a slight secondary fall after a period of 30 minutes. 4 p.m. Dog vomits repeatedly. 5 p.m. Dog vomits repeatedly; salivation and diarrhea marked. 10 p.m. Death.

Autopsy.—Typical picture of acute poisoning by duodenal loop fluid.

The preceding experiments (Dogs S-39 and O-74) show that this poison can be obtained from a mixed solution by alcoholic precipitation. The precipitate when extracted with water yields all of the toxic substances, and the amount of the poison must be very small indeed.

Duodenal Loop Fluid—Toxic Protease.

Loop fluid (1,100 cc.) was collected from six dogs. All these dogs had closed loops of varying length including the duodenum and upper part of the jejunum, varying from 20 to 30 inches in length. The loops were isolated by means of tapes which occluded the lumen of the intestine, and a gastro-enterostomy was performed a few inches below the lower ligature. This mixture was preserved a few days with chloroform and toluol at room temperature. It was then incubated for 48 hours at 38° C. and again allowed to stand at room temperature for 2 weeks.

1,000 cc. of the mixture were precipitated with 5 liters of 95 per cent alcohol at room temperature and allowed to stand at room temperature for many weeks with occasional shaking.

100 cc. were tested by preliminary experiments to determine the amount of toxic substance and some of its reactions. The fluid was boiled over a free flame giving a moderately voluminous precipitate. The supernatant fluid was poured

off and the coagulum extracted again with boiling water which was added to the decanted fluid, making about 120 cc. in all. This fluid was filtered through paper. It reacted faintly alkaline to litmus. Half saturation with ammonium sulphate gave a moderate flocculent precipitate. Five volumes of 95 per cent alcohol gave about the same amount of precipitation. Complete saturation with ammonium sulphate gave but a little more precipitate than did half saturation. Saturation with sodium chloride gave less precipitate than alcohol or ammonium sulphate.

Dog 15-21.—Normal adult spaniel, female; weight 23 pounds.

Mar. 31, 11 a.m. Intravenous injection of 100 cc. of the clear duodenal loop fluid which had been tested above. Temperature at end of the injection 99.4° F. 12 m. Dog not intoxicated, vomited little mucus. No feces passed. Rectal temperature 99.7° F. 12.30 p.m. Dog is vomiting again and passed one semisolid stool; pulse is fair. 1.30 p.m. Dog is vomiting again but is not severely shocked. 3 p.m. Dog is quite sick. Pulse is of a low tension. Dog vomited bile-stained mucus. Temperature 102.5° F. Next day dog appeared normal.

This experiment shows that one-eleventh of the entire fluid did not contain a lethal dose of poison and probably contained about one-half or less than one-half of a lethal dose. We may assume with some justification that the remaining 1,000 cc. of duodenal loop fluid contained from 3 to 6 times a lethal dose for a dog of 15 to 20 pounds' weight.

Duodenal loop fluid (1,000 cc.) was precipitated with 5 liters of 95 per cent alcohol, standing at room temperature for 12 weeks. The precipitate was not abundant. It was thrown upon a paper filter and partially dried. The precipitate was then washed from the filter paper with about 550 cc. of hot distilled water. The mixture made faintly acid to litmus with acetic acid and boiled over the free flame gave an abundant flocculent precipitate which was removed by centrifugalization. The supernatant fluid was poured through a paper filter, giving a total amount of 350 cc. of pale canary yellow, slightly opalescent fluid.

Of this clear filtrate 250 cc. were precipitated with an equal volume of saturated solution of ammonium sulphate and allowed to stand at room temperature. The abundant precipitate appeared slowly and was finally thrown down by the centrifuge. The supernatant fluid was decanted and the precipitate dried between filter paper. This material was dissolved in water to about 125 cc. and heated to boiling over a free flame. It gave a fine flocculent precipitate which was thrown down in the centrifuge, and amounted to about 0.25 the volume of the original ammonium sulphate precipitate. This represents almost the last trace of albumin. The supernatant fluid is almost water-clear and is faintly acid to litmus, but it gives a good precipitate on half saturation of ammonium sulphate. The volume of this solution equals 115 cc., and 65 cc. of this fluid given intravenously in Dog 15-50 cause fatal intoxication in 3½ hours (see also Dog 15-51).

Dog 15-50.—Small black and tan, adult, male; weight 13 pounds.

June 11, 12 m. Kymograph observation with ether anesthesia. Purified loop fluid (65 cc.), described above, given intravenously. Blood pressure was very little influenced by this injection, but the heart was somewhat slowed and respira-

tion was considerably accelerated. Muscular twitchings were quite conspicuous at the end of the injection and this was due probably to the ammonium sulphate remaining in this solution. 12.45 p.m. Dog was removed from kymograph and out of ether. Passed one soft stool and vomited once. 2.30 p.m. No vomiting, but considerable salivation; pulse is weak and dog is cold and looks badly shocked. 3.15 p.m. Blood pressure very low; no vomiting and no diarrhea. Dog in semistupor and very cold. 3.45 p.m. Condition the same; temperature 96.8° F. 4 p.m. Death with autopsy immediately.

Autopsy.—Blood examination showed a considerable excess of antithrombin, as is usual after injection of the crude duodenal material; thorax and the lungs are normal; liver is deep purple and remarkably engorged, otherwise normal. Spleen is greatly enlarged and the splanchnic vessels are very conspicuous. Stomach shows a pale pylorus and cardia and a deep pinkish mucosa in the middle zone. Mucus and fluid very abundant in stomach and small intestine. Duodenum shows a deep purple red velvety mucosa coated with large amounts of mucus and contains much fluid. This color fades to a pink color in the ileum. The colon shows a mottled pinkish red mucosa. This picture is typical of acute poisoning by a large dose of duodenal loop fluid or fluid obtained from above an intestinal obstruction.

The remaining purified duodenal fluid (50 cc. in amount) which had been precipitated with alcohol and then with ammonium sulphate, dissolved in water, boiled, and filtered, was next dialyzed against 0.8 per cent sodium chloride in a collodion tube. This gave a turbidity and slight increase in volume. After dialysis for 18 hours the fluid was boiled and a small precipitate removed by the centrifuge. Filtration through paper gave an odorless, water-clear, but slightly opalescent fluid which was used for further tests. Dialyzed fluid (5 cc.) was precipitated in an Esbach tube with Tsuchiya's reagent (phosphotungstic acid 1.5, hydrochloric acid 5, alcohol 100 parts). This gave 1 gm. per 1,000 cc., as read from the tubes. This represents about 1 mg. per cc. of fluid. The dried weight was estimated carefully for 2 cc. and equalled 1.04 per cent. Nitrogen determination showed that the ammonium sulphate had not been completely removed by dialysis. The dried weight by calculation, allowing for 0.8 per cent sodium chloride, gives a maximum of 120 mg. of material in 50 cc. of this solution. The Esbach determination gives 50 mg. per 50 cc. The first estimation by the dried weight is probably too high, but more accurate than the Esbach. A fairly safe estimate of 100 mg. of proteose in this 50 cc. of fluid may be made. This fluid (50 cc.) was injected into Dog 15-51 with fatal result.

Dog 15-51.—Small fox-terrier, female; weight 15½ pounds.

June 14, 12 m. Kymograph observation with ether anesthesia. Solution of pure proteose (50 cc.) from duodenal loop fluid given intravenously. This caused no reaction on the blood pressure or pulse beat. 12.30 p.m. Animal seems quite unaffected by injection. Removed from kymograph. 1.30 p.m. Dog recovered and seems normal; walks about cage. 4 p.m. Dog is prostrated and greatly shocked; slow deep respiration, vomiting, and diarrhea noted. Temperature 104.2° F. Pulse tension poor. 10 p.m. Dog appears shocked and cold.

June 15, 9 a.m. Dog found dead and cold; much fluid feces in cage.

Autopsy.—Thorax, heart, and lungs normal; spleen and liver only moderately congested; stomach contains blood-tinged fluid and shows moderate congestion of the mucosa. Duodenum contains a good deal of mucus and fluid, and the mucosa is pinkish red and definitely engorged with blood. Jejunum and ileum show a similar picture and contain much watery fluid with mucus. This picture is characteristic of moderately acute intoxication and this dog was given just about a minimal lethal dose of the poison, causing death in about 15 hours with characteristic delayed symptoms of shock, associated with vomiting and diarrhea. It is probable that this dog received approximately 100 mg. of purified proteose obtained from duodenal loop fluid.

This isolation of a primary proteose from the duodenal loop fluid is well established by the above experiments. One liter of duodenal loop fluid contained several lethal doses of the characteristic poison. The toxic substance was first precipitated by five volumes of 95 per cent alcohol and the precipitate dissolved in water. This solution made faintly acid and boiled gave an abundant albuminous precipitate which was removed. The solution was treated with equal parts of a saturated solution of ammonium sulphate which gave a white flocculent precipitate. This precipitate was dissolved in water and the solution again boiled to remove all albumin. This clear filtrate contained the characteristic poison, and 65 cc. poisoned a dog fatally in four hours (Dog 15-50). The rest of this clear filtrate was dialyzed for eighteen hours, again boiled and filtered, giving a water-clear, slightly opalescent fluid. This fluid (50 cc.) contained about 100 mg. of proteose and was sufficient to poison fatally a dog weighing fifteen pounds (Dog 15-51).

This method of isolation removes practically all substances from the duodenal loop fluid mixture with the exception of the primary proteoses. This pure substance gives the identical toxic symptoms noted after injection of the crude duodenal loop fluid. Moreover, the alcoholic extract or filtrate is non-toxic and the bulk of the toxic material can be isolated and purified without great loss. This is strong, if not conclusive, evidence that the essential toxic substance has been isolated from the duodenal loop fluid and that the toxic substance is a primary proteose.

DISCUSSION.

Many interesting points concerning proteose intoxication have been brought out by the work of Chittenden (14) and his coworkers. These investigators show that certain proteoses may stop urinary secretion without much blood pressure reaction. This toxic proteose of intestinal obstruction may cause a sudden stoppage in urinary secretion and no primary blood pressure effect. The fall in blood pressure may not appear until hours after the injection when the symptoms of shock are in evidence.

Some proteoses may be changed before their excretion in the urine,—a primary proteose being excreted as a secondary proteose. It is very important to emphasize that when these proteoses escape from the body they do so by way of the kidneys. It has been shown by Hartwell and Hoguet (5) and others that administration of fluid is of benefit in the intoxication of intestinal obstruction, and it is highly probable that the diuresis aids in the elimination of the poison which may escape in part by way of the urine.

We have attempted to isolate this toxic proteose from the urine of dogs with intestinal obstruction and closed loops of intestine, and following injection of the toxic proteose. There are obvious difficulties in this work when we consider the small amount of the poison (100 mg.) which may produce fatal intoxication. Its injection in considerable amount is attended by cessation of renal excretion. It is not sufficient to state that a proteose appears in the urine after the intravenous injection of this toxic proteose or after an experimental closed intestinal loop. Various procedures may cause albuminuria in a dog, and this is often associated with a definite proteosuria (as shown by Hammarsten's method) without any evidences of intoxication. The determination of a proteose in small amounts in a dog's urine has no special significance. We have attempted to isolate a toxic proteose from dog's urine after obstruction or closed intestinal loops, but so far without success. It is possible that this specific primary proteose may be modified in some way before its excretion in the urine.

Animals react differently to the proteose groups of poisons. Underhill (15) and others point out that dogs are susceptible to pro-

teose intoxication, whereas cats and rabbits are very resistant. Davis and Morgan (16) have shown that cats are very resistant to the poison found in the closed intestinal loops of dogs or cats. Moreover, cats will survive a closed intestinal loop of a certain type much longer than a dog under parallel conditions.

Dogs react constantly to this proteose obtained from closed intestinal loops. The intensity of the clinical symptoms of salivation, vomiting, diarrhea, and prostration depend upon the amount of poison injected. The blood may show a great increase in antithrombin, which will delay or completely prevent clotting *in vitro*. This reaction is variable, but usually follows intravenous injection of a variety of proteoses. In fatal poisoning the splanchnic engorgement is the striking feature. The liver and spleen are swollen and purple. The mucosa of the duodenum especially, but also of the whole intestine, is greatly congested and may be a velvety purplish red color.

It is to be emphasized that other poisons can cause this poisoning with peculiar splanchnic paralysis and engorgement in dogs. It has been noted with various putrefactive poisons, toxic bases or amines (Faust), also after large doses of adrenalin, and in fatal anaphylaxis. It is not safe to draw conclusions about the nature of a given poison solely from its physiological action, but chemical tests must be added.

An interesting experiment has been performed by Murphy and Brooks (7), who isolated the gall bladder after introducing diluted intestinal contents. The dogs died with symptoms of intoxication, and the contents of their gall bladders were toxic to normal dogs, giving the usual picture of proteose intoxication. It is possible that growth of bacteria in association with the mucosa of the gall bladder can produce a toxic proteose. More work must be done, however, to establish this important point. On the other hand, it is possible that some putrefactive poison is responsible, because such poisons and toxic proteoses give similar reactions when given intravenously in dogs.

It is to be emphasized that the group of putrefactive poisons is not found in closed intestinal loops, and the only toxic element is a primary proteose.

Proteose intoxication causes a striking rise in the incoagulable nitrogen of the blood, which may double in amount in a period of three hours. Intestinal obstruction or a closed intestinal loop may cause a great rise in the non-coagulable blood nitrogen, which may even rise to ten times normal. This seems to depend within limits upon the severity and acuteness of the intoxication. Detailed experiments will be published in the near future, and the incoagulable nitrogen may prove to be of clinical value from the standpoint of diagnosis and prognosis.

SUMMARY.

A definite intoxication develops as a result of a closed intestinal loop and toxic material accumulates in the closed loops. Much evidence has been submitted to show that this loop poison causes the intoxication observed after producing a closed intestinal loop. Sufficient evidence has been presented to prove that the essential poison is present in these closed intestinal loops, and usually in concentrated form.

Chemical study of the contents of closed intestinal loops shows that a single substance or group of substances possesses toxic properties. This resists autolysis and pancreatic and ereptic digestion. It is thrown out of solution by five volumes of alcohol or by half saturation with ammonium sulphate. It is readily soluble in water and is not injured by boiling. It is not removed by dialysis. The method of isolation excludes practically all substances except primary proteoses. The characteristic resistance to digestive enzymes suggests a heteroproteose.

Proteose intoxication in dogs gives a picture identical with that described after poisoning with intestinal loop fluid: early salivation and vomiting, followed by diarrhea and prostration, fall in temperature and blood pressure, and finally death in collapse. Autopsy shows essentially a splanchnic paralysis and remarkable engorgement of liver and spleen, but especially of the mucosa of the duodenum and small intestine. The blood shows great concentration due to loss of fluid and may remain incoagulable because of an excess production of antithrombin.

Proteoses escaping from the blood are excreted in the urine. This toxic proteose concerned in intestinal obstruction has not yet been isolated in the urine, but may be excreted by the kidneys. This probably explains the clinical improvement and lessened intoxication noted after transfusion.

Experimental evidence points to a primary proteose as the essential poison concerned in the intoxication of closed intestinal loops and intestinal obstruction.

BIBLIOGRAPHY.

1. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 286.
2. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 307.
3. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1914, xix, 144.
4. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1914, xix, 166.
5. Hartwell, J. A., and Hogue, J. P., *Jour. Am. Med. Assn.*, 1912, lix, 82.
6. Draper, J. W., *Jour. Am. Med. Assn.*, 1914, lxiii, 1079.
7. Murphy, F. T., and Brooks, B., *Arch. Int. Med.*, 1915, xv, 392.
8. Hartwell, J. A., Hogue, J. P., and Beekman, F., *Arch. Int. Med.*, 1914, xiii, 701.
9. Albeck, V., *Arch. f. klin. Chir.*, 1902, lxv, 569.
10. Faust, E. S., *Arch. f. exper. Path. u. Pharmacol.*, 1904, li, 248.
11. Dale, H. H., and Laidlaw, P. P., *Jour. Physiol.*, 1910-11, xli, 318.
12. Murphy, F. T., and Vincent, B., *Boston Med. and Surg. Jour.*, 1911, clxv, 684.
13. Kukula, *Arch. f. klin. Chir.*, 1901, lxiii, 773.
14. Chittenden, R. H., Mendel, L. B., and Henderson, Y., *Am. Jour. Physiol.*, 1898-99, ii, 142.
15. Underhill, F. P., *Am. Jour. Physiol.*, 1913, ix, 345.
16. Davis, D. M., and Morgan, H. S., *Bull. Johns Hopkins Hosp.*, 1914, xxv, 39.

INTESTINAL OBSTRUCTION.

VI. A STUDY OF NON-COAGULABLE NITROGEN OF THE BLOOD.

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This communication deals with analyses of the blood in intestinal obstruction, intestinal closed loops, and other acute intoxications. The tables give figures for non-coagulable nitrogen, urea nitrogen, and in some instances the total nitrogen partition in the blood. Our interest in this study of the blood was aroused by a communication of Tileston and Comfort (1), who in a large series of human cases reported three cases of intestinal obstruction with very high non-coagulable nitrogen. Since that time we have studied the blood of various experimental animals which were being observed in connection with other experimental work.

We found that most cases of intestinal obstruction, especially with signs of acute intoxication, showed a high non-coagulable blood nitrogen, and it seemed possible that this factor might be of value in diagnosis and especially prognosis of acute abdominal conditions. We are now convinced that this non-coagulable nitrogen determination is of value in various acute intoxications. If the reading is high, one may assume a dangerous grade of intoxication, but, on the contrary, one may not assume that a low reading gives evidence of slight intoxication, because a fatal outcome may be associated with a low reading. It is of considerable value to know that the non-coagulable nitrogen of the blood may show high readings in other conditions besides renal disease.

On the other hand, determinations of the blood urea alone are of somewhat less value in studying the retention products in the blood in these conditions. In our experimental animals the blood urea

has varied from less than 30 per cent to more than 80 per cent of the total non-coagulable nitrogen, and, while a high urea reading is the rule, the variations in the urea curve and the curves of the other non-coagulable nitrogenous substances are so great that the urea reading is a somewhat unreliable index of the extent to which non-coagulable nitrogenous substances have accumulated.

Our results are given below in the form of tables and some clinical and experimental data are given in addition, but it is not needful to give the entire experimental data at this time. Usually one experiment in each table is given in some detail as an example of the group.

Methods.

Dogs were used in most experiments. A few cats were used, and one human case was included. All operations on animals were done under surgical anesthesia with the usual surgical technique.

The blood was taken in a glass syringe from the jugular vein and used for the various tests. When the blood was obtained at the time the animal was killed, it flowed from a cannula in the carotid. A few samples taken *in extremis* were obtained after anesthesia by opening the thorax and aspirating direct from the heart.

Non-coagulable nitrogen is determined as follows: With a volumetric pipette 10 cc. of blood are added to 65 cc. of distilled water, and 5 cc. of a 1 per cent sodium oxalate solution. The mixture is brought to a boil, gently rotating the flask while heating, and is then faintly acidified with acetic acid. A few crystals of sodium sulphate are added, the mixture is shaken, and, after the addition of 20 cc. of a 1 per cent solution of uranium acetate, is thoroughly shaken again and filtered. Nitrogen estimations by the Kjeldahl method are done in duplicate on 30 cc. samples of the clear filtrate,—each representing 3 cc. of blood,—and the non-coagulable nitrogen calculated for 100 cc. Creatinine and creatine are determined by the method of Folin (2) with a standard of creatinine zinc chloride. Urea is determined by the method of Marshall as modified by Van Slyke and Cullen (3). Uric acid is determined by the method of Benedict (4).

EXPERIMENTAL OBSERVATIONS.

Simple Obstruction, Recovery, and Second Operation.

Dog 15-12.—Mongrel, female; weight 40 pounds.

Mar. 8. A simple obstruction was made in the middle of the small intestine, and a small piece of tape was fixed about the small intestine to occlude its lumen but not to injure the intestinal wall.

Mar. 9. Dog quiet. Temperature 38.5°C. Slight vomiting.

Mar. 10. Dog shows usual picture of obstruction with vomiting. Temperature 38°C. Weight 38 pounds.

Mar. 11. Condition the same. Intoxication not striking. Weight 36 pounds.

Mar. 12. Condition the same. Weight 34 pounds. Late in the day the dog showed intoxication and small pulse. Infusion of 1,000 cc. of Locke's solution given in the jugular. Blood taken before and after infusion. The infusion caused clinical improvement.

Mar. 13. Dog still vomiting; the intoxication is not severe. Temperature 38.5°C. Weight 35.5 pounds. The marked drop in non-coagulable nitrogen is a striking feature of this experiment, and is difficult to explain, although the diuresis and replacement of lost fluid may be important factors. 1 p.m. Ether anesthesia and laparotomy. The obstruction was removed easily, and the intestinal wall closed over by sutures. At the end of the operation intravenous infusion of 1,000 cc. of Locke's solution. Dog made good recovery from operation.

Mar. 14. Dog vomited a little and refused food.

Mar. 15. Vomiting continues, and animal eats a little. Temperature 38.7°C. Weight 34 pounds. Given 1,000 cc. of Locke's solution intravenously.

Mar. 16. Much improvement; dog takes food; no vomiting. Weight 35 pounds.

Mar. 17 and 18. Continued improvement.

Mar. 19. Improvement continues; dog has passed feces.

Apr. 8. Dog appears normal. Second operation to establish a drained loop of duodenum including about 8 inches of the first part of the jejunum; distal portion of loop pulled through a puncture wound in left rectus; duodenum closed below pancreatic duct and gastrojejunostomy done.

Apr. 9. Dog sick, vomiting. Pulse weak. 3 p.m. Severe intoxication; much chocolate colored vomitus. Ether anesthesia. Killed.

Autopsy.—Thorax, heart, and lungs normal; abdominal viscera are normal; peritoneum is clean; gastro-enterostomy is patent; stomach shows slight engorgement of mucosa. Duodenum between pylorus and point of section shows a swollen and congested mucosa. It contains a blood-stained fluid. Jejunum below gastro-enterostomy shows engorgement of its mucosa very like that noted after lethal injection of a toxic protease. The ileum and colon show congestion of their mucosa to a less extent. The loop contains chocolate-red, slimy material; the mucosa shows no ulceration, but is congested and deep red; there are some submucous ecchymoses.

This experiment is of interest because of the recovery from the first obstruction. An obstruction was produced in the middle of the small intestine by means of a tape. On the 6th day the tape was removed at a second operation, and the dog returned to a normal

TABLE I.

Dog 15-12. Simple Obstruction, Recovery, and Second Operation.

Day after operation.	Time.	Non-coagulable nitrogen.*	Remarks. Mongrel, female; weight 40 lbs.
		mg.	
2	2.00	23	Vomited once. Pulse good. Temp. 38.5°C.
3	11.00	24	Dog vomiting. Condition good. 38 lbs.
4	4.00	68	Dog vomiting frequently. 36 lbs. Temp. 38°C.
5	11.00	119	Dog vomiting. Pulse good. Feces in cage. 34 lbs. Temp. 38°C.
5	2.50	131	Dog prostrated. Pulse tension low. Infusion of 1,000 cc. of Locke's solution.
5	3.20	117	Blood after infusion. Clinical improvement.
6	11.30	31	Dog improved. Vomiting less.
2nd operation.	1.00	42	Laparotomy and removal of obstruction. Infusion of 1,000 cc. of Locke's solution. Blood at end of infusion.
7	11.00	36	Vomits occasionally. Refuses food.
9	10.00	26	Dog improving. 35 lbs.
11	4.00	28	Dog improving rapidly. 35.5 lbs. Temp. 38.4°C.
12	11.00	31	Dog passed feces. Seems quite well.
32	—	—	Normal.
3rd operation.	3.00		Drained loop of jejunum. Long operation.
33	12.00	56	Dog much intoxicated and vomiting. Urea nitrogen 31 mg.
33	3.00	71	Animal acutely sick. Killed. Amino nitrogen 9.3 mg.

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

condition. The third operation was performed, and about 18 inches of the lower duodenum and first portion of the jejunum were isolated as a drained loop opening in the left side. A gastro-enterostomy was performed. The animal showed grave intoxication with much vomiting and death in 24 hours. There was no peritonitis but some interference with the blood supply of the drained loop.

Table I shows the fluctuation in the blood non-coagulable nitrogen. There was a steady rise to a maximum of 131 mg. per 100 cc. of blood on the 5th day. The dog was given an infusion, and the following day the non-coagulable nitrogen had dropped to 31 mg. One is tempted to assume as a simple explanation that the replace-

ment of fluid and the consecutive diuresis sweep out these substances from the blood. This undoubtedly does occur, but the story is not quite as simple as this. For example, in other experiments with a grave intoxication a transfusion may not affect in the least the level of blood non-coagulable nitrogen, in spite of a striking diuresis. There is no evidence to point to lack of eliminative power of the kidneys as a factor.

TABLE II.
Simple Obstruction.

Dog No.	Day after operation.	Non-coagulable nitrogen.*	Urea nitrogen.*	Weight.	Temp.	Remarks.
		mg.	mg.	lb	°C.	
15-20	6	48	—	15.3	38.5	No vomitus. Dog quiet.
	7	33	—	14.5	38.4	Condition the same.
	8	38	—	14.3	39.1	" " "
	9	40	19	13.8	38.5	Little vomitus, intoxication moderate. Killed.
15-26	1	77	—	—	—	Blood after operation.
	3	46	34	42.8	39.2	Vomiting and sick.
	4	60	35	41.3	39.2	Condition the same.
	5	56	40	40.8	39.3	" " "
	6	60	39	40.0	39.1	" " "
	7	94	58	39.0	39.6	Dog weak.
	8	79	42	—	—	Dog seems better. Killed. Slight chronic nephritis.
16-45	1	34	15	36.0	—	Blood before operation.
	3	52	28	34.0	39.7	Vomiting large amounts.
	4	59	21	33.0	39.3	Vomiting.
	6	90	43	—	—	Dog acutely sick. Killed.
16-56	1	—	22	22.0	—	
	4	—	22	19.0	38.0	Dog vomiting since operation.
	5	63	21	—	37.7	11 a.m. Animal in fair condition. Proteose injection. Blood before injection.
	5	81	27	19.5	—	11.10 a.m. Recovered from proteose injection in 6 hrs. Died in night. Blood after injection.

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

Simple Obstruction for 5 Days. Toxic Death.

Dog 16-45.—Shepherd, mongrel, female; weight 36 pounds.

Nov. 10. Simple obstruction in the middle of the small intestine by means of complete section and an inversion of cut ends.

Nov. 11 and 12. Dog is vomiting, and there is a steady loss in weight.

Nov. 13. Much thin yellow vomitus. Temperature 39.3°C. Weight 33 pounds.

Nov. 15. 9 a.m. Dog is severely intoxicated. Ether anesthesia. Killed. The readings for non-coagulable nitrogen and urea nitrogen are given in Table II.

Autopsy.—Thorax, lungs, and heart normal. The viscera show nothing of interest except a slight congestion. The peritoneum is dry and clean. There are a few fibrinous adhesions about the site of obstruction. Intestinal tract below obstruction is collapsed and mucosa normal. The intestine above the obstruction shows only slight dilatation. There is no congestion of the mucosa and no ulceration. The stomach shows some congestion of the cardia. The material above the obstruction consists of thin yellow material, like pea-soup. 160 cc. in amount. Kept for further study.

The protocol of Dog 16-45 gives a typical story of an uncomplicated, untreated case of simple obstruction of the middle of the small intestine. Vomiting is constant, but the grave intoxication does not appear until the 5th day, when the non-coagulable nitrogen is found to be high—90 mg. per 100 cc. of blood. The autopsy findings are characteristic, and the peritoneum is clean.

Table II shows a considerable variety of non-coagulable nitrogen readings. The last three experiments show a definite rise in non-coagulable nitrogen above normal, and this is the rule. The first experiment, however, is a good example of a dog which maintained practically a normal level in spite of a prolonged obstruction. It should be kept in mind that this dog had a resistance considerably above normal, consequently was not as acutely intoxicated, and was killed 8 days after operation while still in fair condition. A similar state of affairs is noted in closed loops of the intestine, and with a slowly progressing intoxication the non-coagulable nitrogen may scarcely rise above its normal level.

This table of simple obstruction experiments gives also the urea nitrogen of the blood. It is noted at once that these intoxications give different urea readings with high non-coaguable nitrogen than is found in chronic nephritis. Urea nitrogen in nephritis usually

constitutes more than 50 to 60 per cent of the total nitrogen, but here it is seen that it often falls below 50 per cent. The residual or undetermined nitrogen in these experiments is constantly high. We suspect that this is a fairly constant feature in various proteose intoxications.

TABLE III.

Closed Loop of Duodenum and Jejunum. Gastro-Enterostomy.

Dog. No.	Day after operation.	Non-coagulable nitrogen.*	Remarks.
15-2		mg.	
	2	41	Condition good, vomiting.
	3	89	" "
	4	113	Grave intoxication; subnormal temperature. Killed.
15-9	1	51	Blood taken at time of operation. Weight 33 lbs.
	2	103	Dog sick, listless, vomiting. Died during night.
15-10	1	20	Blood at operation. 23 lbs.
	2	35	Dog toxic. Pulse good.
	3	70	Dog in fair condition. Intoxication moderate.
	4	135	Dog toxic. Pulse weak. Killed.
15-14	1	40	Blood before operation. 30 lbs.
	2	33	Dog vomiting. Slightly toxic.
	3	37	" " Moderately toxic. 28.5 lbs.
	4	53	Little vomiting. Condition the same.
	5	55	11 a.m. Dog sick. Muscular tremors. 26.5 lbs.
	5	65	2.30 p.m. Killed. Amino nitrogen 5.7 mg. per 100 cc.

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

Long Loop of Duodenum and Jejunum. Gastro-Enterostomy.

Dog 15-10.—Long haired mongrel, male; weight 23.5 pounds.

Mar. 3. Isolated a long loop including lower half of duodenum and upper part of the jejunum; occlusion was effected by means of tapes applied as usual; gastro-enterostomy performed.

Mar. 4. Dog intoxicated. Temperature 38.5°C.

Mar. 5. Condition unchanged.

Mar. 6. Dog very sick; much vomiting. Pulse pressure low. Slow respiration. Temperature 37.3°C. Ether anesthesia and bleeding from carotid, which showed an arterial pressure scarcely above the usual venous pressure.

Autopsy.—Thorax normal. Peritoneum is clean and dry, except for a few bits of fibrin close to the site of operation. Liver shows a little congestion.

Other organs are normal. The loop is completely isolated from the intestine it contains about 50 cc. of creamy, white, syrupy material having the characteristic odor. The mucosa shows a very slight amount of congestion, no ulceration, and is intact throughout. Under the microscope it appears normal. Loop fluid preserved for further study.

The protocol of Dog 15-10 (Table III) is a good example of uncomplicated closed intestinal loops of a certain type. It will be seen that the non-coagulable nitrogen of the blood rises regularly with the intoxication developing under these conditions. It must not be forgotten that these loops include the lower half of the duodenum and much of the upper jejunum. The ends of the loop are closed by tapes, and the contents of the upper duodenum must be forced back into the stomach where they gain access to the jejunum by a posterior gastrojejunostomy. This experiment, therefore, causes an obstruction to the first half of the duodenum, and this is of no small importance, as is seen on comparing these experiments with other closed loops of a different sort in Table V. We intend to take up this point in another communication.

TABLE IV.

Closed Loop of Jejunum. Gastro-Enterostomy.

Dog No.	Day after operation.	Non-coagulable nitrogen.*	Remarks.
		mg.	
15-29	2	23	Blood at end of infusion. Intoxication definite. Infusion of 1,000 cc. of 1% dextrose solution. Weight 35 lbs.
	4	35	Vomiting. Pulse fair.
	5	39	Dog weak. Blood taken before infusion. 1,000 cc. of 10% dextrose. 32 lbs.
	6	98	5 p.m. Infusion of 1,000 cc. of 10% dextrose. 11 p.m. Animal moribund. Killed. Loop rupture.
15-45	4	30	Dog slightly intoxicated. 22 lbs.
	5	76	10 a.m. Dog about the same.
	5	115	5 p.m. Dog looks sick. Pulse weak. Killed. Early peritonitis.
15-19	1	31	Blood at end of operation. 22 lbs.
	2	62	Dog very quiet. Died during night. Acute intoxication.

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

Long Loop of Jejunum with Rupture and Peritonitis.

Dog 15-29.—Adult spaniel, male; weight 35 pounds.

Apr. 23. Isolated a long loop including the lower portion of the duodenum and about 3 feet of the jejunum, using tapes. Gastrojejunostomy performed as usual.

Apr. 24. Dog is sick. There is much dark brown vomitus. Pulse poor, and for this reason infusion of normal salt solution plus 1 per cent dextrose was given intravenously, followed by much improvement.

Apr. 25. Intoxication not striking.

Apr. 26. Much vomiting. Weight 33 pounds. Temperature 38.5°C.

Apr. 27. Dog seems sick. Weight 32 pounds. Temperature 38.4°C. 12 m. Infusion of 1,000 cc. of 10 per cent dextrose. 5 p.m. Dog not much improved. Given a second infusion of 1,000 cc. of 10 per cent dextrose.

Apr. 28. Much vomiting. Pulse tension low. Temperature 38.4°C. Weight 31.3 pounds. 5 p.m. Infusion of 1,000 cc. of 10 per cent dextrose. Dog is vomiting constantly. 11 p.m. Dog very sick. Ether anesthesia. Killed.

Autopsy.—The peritoneal cavity contains 500 cc. of blood-tinged loop fluid of the usual appearance. The peritoneum is specked with ecchymoses, but there is no fibrin. Rupture took place at the site of the lower ligature, which cut through the wall of the intestine. Upper ligature is tight. Death took place promptly from absorption of the toxic material poured from the loop into the peritoneum. The organs show some congestion associated with intoxication by means of loop fluid. Loop is empty. It shows congestion and some areas of submucous hemorrhage due undoubtedly to acute distention.

The protocol of Dog 15-29 (Table IV) shows the same rise in non-coagulable blood nitrogen noted in the closed intestinal loop experiments of Table III. These experiments show various complications met with in these loop experiments,—peritonitis, rupture of loop, and overwhelming intoxication. When the intoxication is very acute and severe, the infusion of normal salt or dextrose solutions will not depress the level of non-coagulable nitrogen in the blood.

Long Loop of Jejunum. Rupture in 20 Days.

Dog 16-22.—Fox-terrier, male; weight 18.5 pounds.

Sept. 28. Isolated a long loop of jejunum; ends of loop sectioned and turned in; the jejunum joined around the loop by end to end anastomosis to establish direct continuity of intestinal lumen.

Sept. 29. Dog looks well; no vomiting. (See Table V for details of temperature, weight, and non-coagulable and urea nitrogen). There was steady loss of weight during the next week with occasional attacks of vomiting; at times the dog eats a little food.

TABLE V.
Long Loop, Jejunum, Ileum.

Dog No.	Day after operation.	Non-coagulable nitrogen.*	Urea nitrogen.*	Weight.	Temp.	Remarks.
		mg.	mg.	lbs.	°C.	
16-20	1	49	9	39.0	—	Before operation. Long loop jejunum.
	1	50	8	—	—	After operation.
	3	60	4	—	40.0	Condition good.
	4	28	7	—	40.0	Dog has vomited.
	5	35	11	37.5	40.1	No vomiting.
	8	24	8	36.8	40.1	" "
	9	22	6	37.3	—	Condition improved.
	10	28	8	37.5	39.8	Dog in good condition.
	11	28	8	36.5	39.7	" " " "
	13	33	12	35.3	—	Dog vomiting and intoxicated.
	14	53	16	—	—	Dog vomiting. Infusion of 1,000 cc. of 7% dextrose solution.
	15	51	18	33.0	39.3	Dog vomiting, weaker. Death 18th day.
16-22	1	22	10	18.5	—	Before operation. Long loop jejunum.
	3	36	8	18.0	39.4	Intoxicated. No vomiting.
	4	—	16	17.5	—	Vomiting, large amount.
	5	35	19	17.3	39.3	Little vomiting.
	7	30	14	15.8	38.9	" " Loss of strength.
	9	36	10	15.5	39.1	No vomiting.
	11	35	12	15.5	39.1	Solid feces.
	14	36	6	15.3	—	Condition the same.
	15	48	9	15.0	—	No vomiting.
	16	43	10	14.5	—	Animal looks intoxicated.
	20	41	20	13.7	38.8	Dog weak. Infusion of 1,000 cc. of 7% dextrose.
	21	64	29	—	—	Dog sick. Killed.
16-37	5	90	21	27.3	38.9	Vomiting for past 3 days. Long loop of ileum.
	6	45	20	27.3	39.1	Dog improved; no vomiting; eating.
	10	53	20	25.8	38.9	Some diarrhea.
	11	—	13	—	—	Dog looks well.
	13	59	21	24.3	—	Condition the same.
	14	57	25	24.0	—	Dog improved.
	15	31	19	23.8	—	Passed solid stool. Eating.
	17	54	32	24.0	38.9	Peristalsis visible in abdomen.
	18	40	19	24.0	39.3	Dog losing ground.
	19	40	9	23.8	38.9	Condition good. Killed.

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

Oct. 14. Dog has passed formed stools. No vomitus. Weight 14.5 pounds. There is evidence of chronic intoxication. Dog was injected with a toxic proteose obtained from ox pancreas; dog was slightly intoxicated by this proteose, while a normal control animal was fatally poisoned in 11 hours by a similar dose.

Oct. 15. Dog looks well; no vomitus.

Oct. 18. Dog is very sick. Ether anesthesia. Killed.

Autopsy.—Thorax, heart, and lungs normal; spleen, kidney, pancreas, etc., normal; liver rather atrophic; no signs of fatty degeneration. Peritoneal cavity contains a good deal of the loop fluid which has escaped from a recent rupture. There is little reaction in the peritoneum as the intoxication was so acute. Stomach and intestine outside of loop show congestion of the mucosa so common in proteose intoxication. Loop is made up of two parts isolated by old adhesions. One-half is slightly collapsed due to escape of fluid into the peritoneum; there is an ulcer in the wall, which has perforated the mucosa; all this portion is somewhat red and swollen, and shows two other ulcers involving the mucosa. The other half of the loop consists of three small coils twisted about its mesenteric attachment; adhesions have caused some constriction of mesenteric vessels giving engorgement of the loop but not infarction; this portion of the loop contains a pale, slate colored fluid with no blood; the mucosa is swollen and engorged but shows no hemorrhage; the walls of the loop are everywhere hypertrophied.

The protocol of Dog 16-22 (Table V) shows a different type of closed intestinal loop with a slowly progressing intoxication and relatively slight changes in the non-coagulable nitrogen of the blood. The loops of the jejunum or ileum are isolated completely by cross section of the gut in two places. The loop is made by turning in the ends of the isolated portion of the intestine or by doing an end to end anastomosis thus forming a circle out of the isolated gut. The continuity of the rest of the intestine is established by means of an end to end anastomosis which gives an unobstructed flow from duodenum to jejunum and ileum. This does away with the obstruction in the first half of the duodenum which is present in the loops isolated by ligature and gastrojejunostomy (Tables III and IV). The difference in severity of intoxication under these conditions is obvious.

The urea nitrogen readings in general show a low percentage value of the total non-coagulable nitrogen. The weight curve shows the gradual loss in body weight due to the chronic intoxication, even in the absence of vomiting.

The immunity to proteose injection (Dog 16-22) shown by a dog with a long standing closed intestinal loop indicates that the presence

of a closed loop of intestine causes a chronic proteose intoxication which gives a certain degree of immunity against poisoning by various foreign proteoses. This point will be taken up in another communication and the experiments will be given in detail.

TABLE VI.

Long Loop, Jejunum, Ileum, Complications.

Dog No.	Day after operation.	Non-coagulable nitrogen.*	Urea nitrogen.*	Weight.	Temp.	Remarks.
		mg.	mg.	lbs.	°C.	
16-42	3	32	13	21.0	39.1	Long loop jejunum, obstruction, peritonitis.
	4	39	15	20.5	39.0	Considerable vomiting.
	5	39	13	20.7	38.8	" " Animal looks sick.
	6	50	22	21.0	37.9	9.30 a.m. No vomitus.
	6	56	39	-	34.5	4 p.m. Dog toxic. Killed.
16-48	4	30	14	19.5	39.0	Long loop jejunum, terminal obstruction, and volvulus.
	6	45	19	19.0	39.2	Condition good. No vomiting.
	8	32	15	19.3	38.8	Condition the same.
	12	46	22	19.5	39.3	" " " Found dead on 13th day.
16-57	1	58	23	18.5	-	Short loop of ileum. General peritonitis.
	6	44	11	15.5	39.3	Dog has been in good condition.
	8	166	51	15.5	39.3	Some vomiting. Animal moribund. Killed.
16-71	1	36	12	27.5	-	Long loop of jejunum. General peritonitis.
	2	36	12	26.3	38.3	Dog in fair condition.
	4	53	31	-	-	Animal moribund. Killed.
16-39	1	-	15	24.5	-	Long loop of jejunum. General peritonitis.
	3	71	18	23.5	38.8	Much vomiting. Pulse poor. Infusion of 1,000 cc. of 5% dextrose. Blood at end of infusion. Death next day.

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

Long Loop of Jejunum. Obstruction. General Peritonitis.

Dog 16-42.—Mongrel, male; weight 22 pounds.

Nov. 6. Isolated a long loop of jejunum by section and inversion of ends; jejunum joined around the loop by means of end to end anastomosis between upper and lower end.

Nov. 8. Dog pretty well. Temperature 39.2°C. Weight 22 pounds.

Nov. 9. Much vomiting.

Nov. 10. Dog seems better. (See Table VI for details.)

Nov. 11. Little vomiting. Temperature 37.9°C. Weight 21 pounds. 4 p.m. Dog is very weak. Temperature 34.5°C. Ether anesthesia. Killed.

Aut. p.s.v. Thorax, heart, and lungs normal. Peritoneal cavity contains many isolated pockets of purulent exudate. A large round worm is free in the peritoneal cavity, evidently having escaped from the loop, which contains numerous similar worms. There are organized adhesions just below the end to end anastomosis causing a sharp kink and probably complete obstruction. Duodenum and jejunum contain the usual obstruction fluid. The loop contains 130 cc. of pale, slate colored fluid with a strong odor. There are numerous live and active round worms in the loop. No point can be found where the worm escaped from the loop. The peritonitis is probably of 1 or 2 days' duration.

The protocol of Dog 16-42 (Table VI) shows some of the complications which may arise in the closed loop experiments. Peritonitis is most common, but volvulus and obstruction may occur. These complications do not modify the picture, and we have good evidence that peritonitis alone may be associated with a high non-coagulable nitrogen. It is of interest to note that we have been able to isolate a toxic proteose from peritoneal exudates, and we believe that this proteose is of considerable importance in explaining the intoxication of general peritonitis. The rise in non-coagulable nitrogen in the blood may be in part due to the proteose intoxication.

Proteose Injection. Rise in Non-Coagulable Nitrogen.

Dog 15-50.—Small black and tan, male; weight 13 pounds.

12 m. (A) Blood non-coagulable nitrogen 33 mg. per 100 cc. of blood; ether anesthesia and intravenous injection of purified proteose obtained from closed intestinal loops. Kymograph observation during slow injection of 65 cc. of solution of proteose which had been reprecipitated first by alcohol and then by half saturation with ammonium sulphate as described previously (5). There was a little fall in blood pressure.

12.45 p.m. After injection (B) blood non-coagulable nitrogen 40 mg. per 100 cc. of blood.

3.15 p.m. (C) Blood non-coagulable nitrogen 80 mg. per 100 cc. of blood.

4 p.m. Death with prostration and subnormal temperature.

Autopsy.—There is the typical splanchnic engorgement due to fatal proteose intoxication. Spleen and liver are swollen and purple. Mucosa of duodenum and jejunum is swollen and deep reddish purple.

Dog 15-51.—Small fox-terrier, female; weight 15.5 pounds.

12 m. (A) Blood non-coagulable nitrogen 39 mg. per 100 cc. of blood. Ether anesthesia and intravenous injection of purified proteose obtained from closed intestinal loops. Kymograph observation during injection of 50 cc. of fluid showed no change in blood pressure.

12.30 p.m. After injection (B) blood non-coagulable nitrogen 32 mg. per 100 cc. of blood.

5 p.m. (C) Blood non-coagulable nitrogen 58 mg. per 100 cc. of blood.

Dog is prostrated; much vomiting and diarrhea.

Died in night.

Autopsy.—The findings are characteristic of proteose intoxication. There is much blood-tinged fluid in the intestines, and the mucosa is red and swollen.

These two experiments (Dogs 15-50 and 15-51) are of considerable interest, and show that acute poisoning with a pure proteose may cause the blood non-coagulable nitrogen to double in amount in 3 hours; for example, a rise from 40 to 80 mg. The proteose used in these experiments was pure, and not over 200 mg. were injected. The addition of this to the blood itself could not be detected by any method in use, and the method used by us causes precipitation of all primary proteoses at least. The great rise in non-coagulable nitrogen obviously must be explained by disintegration of body or tissue protein. This is of importance in explaining the high non-coagulable nitrogen associated with the closed loops of intestine or intestinal obstruction. In both instances we are dealing with a proteose intoxication, and we believe that much of the increase in blood non-coagulable nitrogen is due to disintegration of the tissues of the body. Catabolism, in other words, must be responsible for much of the non-coagulable nitrogen rather than retention.

Tables VII and VIII give the nitrogen partition of the total non-protein nitrogen of the blood expressed in mg. per 100 cc. of blood. One control nephritis gives a residual nitrogen of 11 per cent and 82 per cent urea nitrogen. A second case (Dog 16-49 in Table VIII) shows a similar picture. This experiment presents isolation of the bladder and implantation of the ureters into the intestine. There

TABLE VII.

Intestinal Loops. Peritonitis and Obstruction.

No.	Non-coagulable nitrogen.			Urea nitrogen		Amino nitro- gen.	Uric acid nitro- gen.	Creatine nitro- gen.	Creatinine nitrogen.	Residual nitrogen.		Remarks.
	mg.	mg.	per cent	mg.	mg.	mg.	mg.	mg.	mg.	per cent		
Dog 16-37	40	9	23	1.4	0.6	0.6	1.5	26.9	67	Long loop of ileum. Killed 19th day.		
" 16-42	56	39	69	4.5	0.6	1.0	5.6	5.3	9	Long loop of jejunum. Peritonitis, obstruction.		
" 16-57	166	51	30	6.0	0.6	0.8	11.0	96.6	58	Long loop of ileum. Peritonitis.		
" 16-71	53	31	58	3.7	1.1	0.8	7.3	10.1	19	Long loop of jejunum. Peritonitis.		
" 16-80	40	28	70	3.9	1.0	0.3	3.1	3.7	9	Long loop of ileum. Peritonitis.		
" 15-45	115	77	60	—	—	0.8	5.9	—	—	Long loop of jejunum.		
Cat 16-7	296	137	46	6.8	2.3	0.7	21.3	127.9	43	Long loop of jejunum and obstruction. Death in 4 days.		
Dog 16-45	90	43	47	5.5	—	—	—	—	—	Simple obstruction. Death in 5 days.		
" 16-82	47	17	36	2.5	1.4	0.2	3.3	22.6	48	Simple obstruction. Killed in 7 days.		
Case 1 (Man)	164	107	65	2.3	0.9	0.3	10.2	35.2	21	Simple obstruction. Pneumonia. Death in 5 days.		

Nitrogen is given in terms of mg. per 100 cc. of blood.

was some obstruction to the outflow of urine and some escape into the peritoneum with an irritant peritonitis and absorption consequent to this. Here there are two factors—retention or lack of elimination plus peritonitis.

The tables show a high per cent of residual nitrogen or a low relative per cent of urea nitrogen. This is true particularly in the severe proteose intoxication with high non-coagulable nitrogen. Similar high readings in cases of nephritis and retention will scarcely show such high percentages of residual nitrogen. For this reason we be-

TABLE VIII.

Bladder Isolation, Chronic Nephritis, Peritonitis, etc.

Dog No.	Non-coagulable nitrogen.			Urea nitrogen.		Amino nitro- gen.	Uric acid nitro- gen.	Creatine nitro- gen.	Creatinine nitrogen.	Residual nitrogen.		Remarks.
	mg.	mg.	per cent	mg.	mg.	mg.	mg.	mg.	mg.	mg.	per cent	
16-49	130	91	70	2.3	0.3	0.4		7.1	28.9	22		Bladder isolation. Urine escape. Peritonitis.
16-61	368	304	82	6.8	2.3	1.0		12.0	41.9	11		Chronic nephritis. Old dog. Hemorrhagic gastritis.
16-62	72	42	58	4.2	1.0	0.5		1.1	23.2	32		Acute distemper.
16-65	36	18	50	2.8	0.3	0.6		5.2	9.1	25		Drained loop of ileum. Subcutaneous abscess.
16-67	40	16	40	1.5	1.8	0.6		3.8	16.3	40		Skin incisions. Mild distemper.
16-69	164	58	38	4.2	1.2	0.9		5.3	94.4	57		General peritonitis.
16-85	67	29	43	3.1	0.4	0.7		3.6	30.2	45		Distemper. Pneumonia.

Nitrogen is given in terms of mg. per 100 cc. of blood.

lieve that the non-coagulable nitrogen determination gives more information than does urea nitrogen, and is of more clinical value in diagnosis and prognosis.

The blood content of amino nitrogen and uric acid nitrogen is low normal, and shows fluctuations within normal limits. Ammonia nitrogen was done in the majority of experiments but the method is inaccurate and the values are probably too high. It seemed best to leave these analyses out of the table and assume that ammonia nitrogen is included in the urea nitrogen. The difference scarcely exceeds 1 mg. at the most.

The creatine fraction is constantly low, but it is of interest to note that the creatinine nitrogen may be very high in many of these proteose intoxications, particularly in intestinal obstruction and closed intestinal loops. There are many readings of more than 5 mg. per 100 cc. of blood, and one experiment (Table VII, Cat 16-7) shows a reading of 21.3 mg. This occurred in a cat with intestinal obstruction plus a closed intestinal loop with a non-coagulable nitrogen of 296 mg. per 100 cc. of blood. The kidneys in all these cases,

unless otherwise noted, were normal. It is to be recalled that all these animals were sick and refused food—in fact the majority were vomiting more or less. A human case of intestinal obstruction and pneumonia shows a very high creatinine value, 10.2 mg., but autopsy showed practically normal kidneys.

Human Intestinal Obstruction. Death in 5 Days.

Case I.—C. K., German, male, 68 years of age.

Past History.—Negative.

Present Illness.—Began Jan. 16, 1916, with indefinite pain. No bowel movements since this time.

Jan. 17. A good deal of epigastric pain with vomiting, which became fecal in type the following day. Patient given castor oil and salts without results.

Jan. 18. Condition unchanged, except that vomiting became more severe.

Jan. 19. Entered hospital. At this time he had marked abdominal tenderness, and was vomiting frequently. Vomitus fecal in odor, watery, with fine brownish precipitate. Blood obtained showed very high non-coagulable nitrogen. Patient given 900 cc. of 6 per cent glucose with sodium carbonate intravenously. Infusion improved condition of patient.

Blood Examination.—White blood cells 18,400; hemoglobin 90 per cent.

3 p.m. Laparotomy and abdominal exploration. A portion of greatly congested intestine, about 10 cm., was found; the wall was edematous. It was placed back in the abdominal cavity. Abdomen closed. Volvulus(?).

Jan. 20. 10 a.m. Blood again obtained, and showed some decrease in non-coagulable nitrogen; vomiting not so marked; considerable fecal matter obtained by means of an enema. Pulse not good. 4 p.m. Blood again obtained; showed rising non-coagulable nitrogen. Given infusion of 750 cc. of 6 per cent glucose intravenously.

Jan. 21. 6 a.m. Patient died in stupor. Blood obtained by cardiac puncture a few minutes after death.

Autopsy performed 5 hours after death.

Anatomical Diagnosis.—Intestinal obstruction; volvulus (?); operation wound for relief of obstruction; early infarction and necrosis of loop of ileum; early serofibrinous peritonitis; bronchopneumonia (pseudolobar) of both lungs; acute hemorrhagic bronchitis; cloudy swelling of viscera.

The abdomen is considerably distended and tense. On incision a small amount of blood-tinged, slightly turbid fluid is found between the coils of an enormously distended small intestine. The loops are somewhat glued together by dry, plastic exudate. The surface of the intestines is somewhat dry and very definitely injected, more especially in the region of the abdominal incision. There are no adhesions except over the spleen. There are no hernial openings to be found in the pelvis or inguinal region. One of the stitches in the abdominal wound has caught

and firmly held a bit of omentum. One segment of the intestine, dark red in color and considerably swollen, measuring about 15 cm. in length, is found close to the liver. The swelling involves the wall of the intestine and the mesentery to a distance of about 5 cm. from the intestinal attachment. There is a clean-cut line of demarcation between the relatively normal but elongated mesentery and the short, inflamed, edematous mesenteric portion close to the intestine. The line of demarcation on the intestine is quite sharp, particularly at the upper end. It appears as though a band, or definite tight constriction, had been drawn about the portion of the ileum including the small part of the mesentery, shutting off a considerable part of the blood supply. This may have been due to a twist of the relaxed elongated mesentery. This was evidently relieved at operation, but the circulation did not establish itself properly owing to tissue injury, and the general appearance was that suggesting early hemorrhagic infarction. The picture, however, was not complete. Careful section of the mesenteric veins showed them to be quite free from thrombi even in their finer branches. There may be some very small thrombi in the smaller branches close to the mesenteric border, but these could not be dissected out.

Lungs.—The right lung weighs 870, and the left 560 gm. The lungs are voluminous, cushiony in their anterior portions; they are heavy and consolidated posteriorly. The pulmonic vessels are clear. The bronchi are intensely inflamed, their mucosa is velvety and purple, and they contain much serous blood-tinged fluid. On section the anterior portions are dry and cushiony. The posterior portions are consolidated and very moist. One can scrape off purulent material. The consolidation involves the posterior portion of the left upper and the greater portion of the right upper lobe and part of the right middle lobe. These areas of consolidation are mottled gray and purplish red. Some of the gray areas are very soft, and creamy material can be scraped off, indicating a beginning resolution of the exudate. This pneumonic process must have been of several days duration—estimated 2 to 3 days.

Kidneys.—Capsule comes off easily leaving a smooth surface, but for two retention cysts. There is one large retention cyst in the upper pole of the right kidney measuring about 2 cm. in diameter. Its wall contains a few thin plaques of calcified material. The kidney parenchyma on section appears normal. The pelvis is normal.

Microscopic Examination. Kidneys.—There are a few hyaline casts in some of the tubules, also a few hyaline scars in the cortex. In general the parenchyma looks normal except for definite cloudy swelling of the epithelium lining the convoluted tubules. There is no epithelial necrosis. The stroma of the pyramids shows a little edema.

Lungs.—The patches of pneumonia show the alveoli filled with an exudate of coagulated serum, mono- and polynuclear cells, fibrin, and enormous numbers of bacteria. In places the bacteria form almost a solid mat of rods showing capsules. The great overgrowth of bacteria is the striking feature of this lung.

Mesentery.—The swollen hemorrhagic area shows a few small recent thrombi, but most of the veins are free. There is extreme extravasation of red blood cells into its stroma. The other portion of the anatomical protocol may be omitted, as it has no bearing on the points under consideration.

TABLE IX.

Human Intestinal Obstruction.

Time.	Non-coagu- lable nitro- gen.*	Urea nitro- gen.*	Urea.	Remarks.
	mg.	mg.	per cent	
2 hrs. before operation.	145	30	20	After infusion of 900 cc. of 1% sodium carbonate and 6% glucose.
18 " after "	76	48	63	After infusion of 400 cc. of 6% glucose.
26 " " "	80	52	60	" " " 750 " " 6% "
46 " " "	164	107	65	Heart blood (see Table VII).

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

The human case (Table IX) is of much interest as the observations on the blood are fairly complete, and autopsy material is available. Clinically it was a clear case of obstruction due to volvulus with partial infarct formation in the intestine and a complicating pneumonia. The non-coagulable nitrogen of the blood was constantly high in spite of transfusion at various times. The elimination of urine was considerable, because of the transfusions, and showed nothing of interest—a mere trace of albumin and an occasional cast.

Table IX shows that there is a rise in urea and drop in non-coagulable nitrogen after the first infusion. We have noted this under the same circumstances in dogs, but are not able to advance an explanation. The high creatinine value (Table VIII) is of interest, as the kidneys are shown to be practically normal. This case gives blood findings identical with those observed in animals under similar conditions, and indicates that our experiments give information which may be of value in the study of human cases.

DISCUSSION.

We believe that it may be assumed as proven that the non-coagulable nitrogen of the blood in the majority of instances is definitely

increased in intestinal obstruction or with closed loops of intestine. With acute intoxication this rise in non-coagulable nitrogen is apt to be more striking and constant. When this rise in non-coagulable nitrogen of the blood does occur, it is a grave sign, and is a clinical index of a severe intoxication even in spite of other clinical evidence to the contrary. But a low non-coagulable nitrogen does not guarantee a mild grade of intoxication. We are convinced that a knowledge of the blood non-coagulable nitrogen is of considerable clinical value in the prognosis of acute abdominal conditions.

It should be kept in mind that the urea nitrogen as well as creatinine nitrogen may show high values in these conditions, and these points may be of value in differential diagnosis. It is established that other conditions besides chronic nephritis may show a marked increase in the creatinine and urea nitrogen of the blood. It should be recalled that "creatinine rises above 2.5 mg. per 100 cc. of blood almost without exception only in conditions with renal involvement" (Myers and Lough (6)). The conditions studied by us show a high creatinine fraction and constitute exceptions to this statement.

It will be noted that the undetermined nitrogen in these experiments is unusually high—more so than in cases of nephritis with high non-coagulable nitrogen. This may be a peculiarity of this type of intoxication as contrasted with simple retention of nitrogenous material, and a study of this point may bring out much valuable information.

Having established the fact that the non-coagulable nitrogen of the blood is much increased in many cases of intestinal obstruction or of closed intestinal loops, we may now ask: Why does not the kidney eliminate these substances immediately? The kidneys are normal in gross and by functional tests in practically all cases. There can be no true retention because of impaired kidney function. There may be two or more factors concerned. We know that in intestinal obstruction the current of fluid is mainly out of the body and by way of the intestinal tract, and it is possible that this favors the accumulation of certain products in the blood stream. The kidneys excrete small amounts of highly concentrated urine.

It is to be remembered, too, that injection of a small amount of a toxic proteose may cause a great rise in non-coagulable nitrogen in

the blood; for example, a rise from 40 to 80 mg. in 3 hours. This cannot be due to lack of elimination, and we must assume destruction of body protein to account for this remarkable change. We may assume that any acute proteose intoxication may be associated with a similar rapid rise in non-coagulable nitrogen in the blood. When we have more information about this point, we may better understand the manner in which the toxic proteoses injure the body and perhaps the various methods of body defense.

General peritonitis is often associated with a definite rise in non-coagulable nitrogen of the blood. How may we explain this observation? It may be argued that paralytic ileus is alone responsible, and this may be true in part. However, we think it important that a toxic proteose can be isolated from the exudate in cases of general peritonitis, and obviously must be absorbed by the host. The proteose intoxication may well be responsible for this change in non-coagulable nitrogen. We hope to report further on this point in the near future.

SUMMARY.

Intestinal obstruction, as a rule, is associated with an increasing amount of non-coagulable nitrogen in the blood. With acute intoxication the rise in non-coagulable nitrogen may be rapid and reach as high as three or even ten times normal. With more chronic intoxication there may be little or no rise in the blood non-coagulable nitrogen.

Closed intestinal loops show exactly the same picture, and, when combined with obstruction, may give very high nitrogen readings.

Acute proteose intoxication due to injection of a pure proteose will show a prompt rise in blood non-coagulable nitrogen, even an increase of 100 per cent within 3 or 4 hours.

These intoxications also show a high blood content of creatinine and urea. The residual or undetermined nitrogen may be very high.

A human case of intestinal obstruction with autopsy presents blood findings exactly similar to those observed in many animal experiments.

Clinically the non-coagulable nitrogen of the blood may give information of value in intestinal obstruction. A high reading means

a grave intoxication, but a low reading may be observed in some fatal cases and gives no assurance that a fatal intoxication may not supervene.

The kidneys in practically all these experiments are normal in all respects.

It is possible that protein or tissue destruction rather than impaired eliminative function is responsible for the rise in non-coagulable nitrogen of the blood in these acute intoxications.

Transfusions of dextrose solutions often benefit intestinal obstruction, and may depress the level of the non-coagulable nitrogen in the blood. Some cases show no change in non-coagulable nitrogen following transfusions and diuresis, and, as a rule, such cases present the most severe intoxication.

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BIBLIOGRAPHY.

1. Tileston, W., and Comfort, C. W., Jr., *Arch. Int. Med.*, 1914, xiv, 620.
2. Folin, O., *Jour. Biol. Chem.*, 1914, xvii, 475.
3. Van Slyke, D. D., and Cullen, G. E., *Jour. Biol. Chem.*, 1914, xix, 211.
4. Benedict, S. R., *Jour. Biol. Chem.*, 1915, xx, 629.
5. Whipple, G. H., Rodenbaugh, F. H., and Kilgore, A. R., *Jour. Exper. Med.*, 1916, xxiii, 123.
6. Myers, V. C., and Lough, W. G., *Arch. Int. Med.*, 1915, xvi, 536.

PROTEOSE INTOXICATION

Intestinal Obstruction, Peritonitis and Acute Pancreatitis



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PROTEOSE INTOXICATION

INTESTINAL OBSTRUCTION, PERITONITIS AND ACUTE PANCREATITIS *

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This communication deals with the etiology of the intoxication which develops in intestinal obstruction, general peritonitis and acute hemorrhagic pancreatitis. It has been demonstrated¹ that the intoxication of intestinal obstruction is due to a primary proteose which may be precipitated by five volumes of 95 per cent. alcohol or by half saturation with ammonium sulphate. It is comparatively easy to isolate the poison from closed loops of the intestine. This proteose is very toxic and 100 mg. may suffice to poison fatally a 15 pound dog.

Peritonitis and pancreatitis have some clinical features in common with acute intestinal obstruction. At times there may be some difficulties in differential diagnosis. We propose to show that the intoxication in these three conditions is due in large part to toxic proteoses. There may well be other substances concerned, but I believe that the proteose is the most important factor in the toxic reaction following peritonitis and pancreatitis.

It may be objected that when a toxic proteose is isolated from a closed loop of intestine this substance is not actually concerned in the intoxication. For example, it cannot be demonstrated in the blood. But when a toxic proteose is isolated from the exudate of a general peritonitis, no valid reason can be given why this substance is not concerned in the associated intoxication. Absorption from the peritoneal

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* From the George Williams Hooper Foundation for Medical Research, University of California Medical School.

1. Whipple, Rodenbaugh and Kilgore: Jour. Exper. Med., 1916, xxiii, 123.

cavity is known to be very rapid, and any soluble substance like a proteose will be absorbed into the blood stream with alacrity. We can show that a peritoneal exudate contains a toxic proteose which is precipitable by five volumes of 95 per cent. alcohol and closely resembles the proteose isolated from closed intestinal loops. It gives the same biologic reaction when injected into animals.

Further, it can be shown conclusively that this proteose is not due to bacterial activity, as a toxic proteose can be demonstrated in a sterile peritonitis caused by turpentine, aleuronat or bile. It seems safe to assume, therefore, that the proteose must be derived from the proteins of the host. In a general peritonitis due to bacteria the same proteose can be isolated, and it is logical to suppose that here too the proteose may be derived from the tissues or tissue proteins of the host.

A sterile hemorrhagic pancreatitis may be produced by the injection of bile into the pancreatic duct. The clinical picture of intoxication under these conditions is familiar and is very like that of acute intestinal obstruction. During the first twenty-four hours after the operation the animal may be killed, the pancreas rapidly ground up in water, the mixture centrifugalized and the supernatant fluid poured into five volumes of 95 per cent. alcohol. This precipitate contains much albumin which can be removed by heat in a faintly acid solution. The slightly opalescent filtrate can be concentrated and tested in animals to show the presence of a toxic proteose. The amount of proteose here is not great as compared with the amount in an intestinal obstruction, but this difference may be due to the great difference in rapidity of absorption. It is possible that the proteose is absorbed from the pancreas almost as rapidly as it is formed. Jobling² has brought indirect evidence to show that there is a proteose intoxication in acute pancreatitis.

Considerable data³ has been published recently to show the rise in noncoagulable nitrogen of the blood in many conditions of intoxication, especially in acute intestinal obstruction. The noncoagulable nitrogen

2. Petersen, Jobling and Eggstein: Jour. Exper. Med., 1916, xxiii, 491.

3. Cooke, Rodenbaugh and Whipple: Jour. Exper., Med., 1916, xxiii, No. 6.

may rise from a level of 25 mg. per hundred c.c. of blood to 100 or even 200 mg. There is no lack of eliminative power in the kidneys to account for this. Acute proteose intoxications due to the injection of a pure proteose into a normal dog may show a rise in noncoagulable nitrogen from 25 mg. per hundred c.c. of blood to 40 or 60 mg. within three or four hours. A similar rise in blood noncoagulable nitrogen may be found in association with general peritonitis, whether sterile or septic, and acute hemorrhagic pancreatitis.

Experiments have been carried out to explain the rise in noncoagulable blood-nitrogen in these conditions — intestinal obstruction (including closed loops of intestine), general peritonitis and acute hemorrhagic pancreatitis — and also in pleurisy and abscess formation (Cooke, Stearns and Whipple, unpublished). Dogs kept in metabolism cages during starvation after four or five days show a constant urinary nitrogen elimination per twenty-four hours. If a small dose of pure proteose is given intravenously there will be a great increase in the urinary nitrogen elimination — perhaps much over 100 per cent. increase per day, and the increase lasts over three to five days. The greatest increase appears usually on the second day after injection and not during the first twenty-four hours as would be expected. This nitrogen must be derived from the tissues of the animal, and it is to be emphasized that the catabolism and increased elimination lasts for days. The rapid rise in blood noncoagulable nitrogen may last only a few hours following the injection and is usually best seen in fatal cases in which the tissue destruction is extreme. But all the evidence points to catabolism of the hosts' tissues due to the proteose intoxication as explaining the high nonprotein nitrogen of the blood.

In like manner, a dog on starvation with a uniform urinary nitrogen excretion will show a great rise in nitrogen elimination if a closed loop of intestine is produced. The rise may be much over 100 per cent. elimination in twenty-four hours, and may last many days. A certain type of simple duodenal obstruction can be produced with which there will be little or no vomiting and no dehydration. In such cases animals may show over 200 per cent. increase in urinary nitrogen elimination per twenty-four hours, and death in

six or eight days may show a blood noncoagulable nitrogen well over 100 mg. per hundred c.c. of blood.

Similar experiments may be performed on dogs with uniform nitrogen excretion when a pancreatitis, peritonitis, pleurisy or sterile abscess has been produced. The same prompt rise in nitrogen elimination will follow the inflammation. It is not to be considered for a moment that this reaction means simply a disintegration of tissue at the site of inflammation and an elimination of the nitrogen from this tissue only. We are dealing with an increase of 2 or 3 gm. of nitrogen per twenty-four hours which can scarcely be accounted for by injury of the local tissue. We assume that the injury of the local tissue gives rise to toxic proteoses which are absorbed into the blood and injure the entire organism, causing among other things a considerable destruction of tissue protein in all parts of the body and the resultant increase in blood noncoagulable nitrogen followed by a great rise in urinary nitrogen.

These toxic proteoses isolated from the intestine, from the peritoneum and from the pancreas have certain biologic reactions in common, but give no specific reactions by which we can differentiate them. They give no anaphylactic reactions in guinea-pigs, no precipitins, no complement fixation. It has been shown⁴ that the blood of dogs injected repeatedly with proteose cannot destroy the toxic proteose, whereas the tissues of such animals can rapidly destroy such proteoses, *in vitro*.

The proteose concerned in the intoxication of intestinal obstruction is resistant to digestion by intestinal mucosa, and pancreatic and tissue ferments. We have not yet determined whether the toxic proteoses concerned in hemorrhagic pancreatitis and general peritonitis possess the same resistance to digestive enzymes.

Any animal injected with one proteose becomes resistant not only to this proteose but also to other proteoses. For example, proteose from human material when injected into a dog will give tolerance to any of the proteoses obtained from the intestine or peritoneum of the dog or cat. This holds for all proteoses tested by us.

4. Whipple, Stone and Bernheim: *Jour. Exper. Med.*, 1914, xix, 144.

It is important to note that dogs with long continued obstruction or closed intestinal loops will survive lethal doses of pure proteose with but few clinical symptoms of intoxication. Dogs recovering from a sterile pleurisy or peritonitis also show a definite resistance or tolerance to subsequent proteose injections. All this evidence strengthens the argument that a proteose intoxication is present in these various conditions.

Other conditions in which inflammation and pus formation or tissue destruction are conspicuous may be considered in which it is possible that toxic proteoses may be concerned. We have made experiments with sterile pleurisy, considered as identical with peritonitis, and with sterile abscess formation, but do not care to report our findings at this time. Infarcts, pneumonia and many other conditions are interesting possibilities which are being studied.

I feel confident that sufficient evidence has accumulated to show that there is a definite proteose intoxication in intestinal obstruction and allied conditions, in general peritonitis, either septic or sterile, and in acute hemorrhagic pancreatitis. I believe that the proteose intoxication is the most important factor in the general intoxication noted in these conditions. The detailed experiments will be published in the near future.

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